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Canada Centre for Mineral and Energy Technology
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Canada

INTERNAL BIOCORROSION OF STEEL PIPES

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INTERNAL BIOCORROSION OF STEEL PIPES

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March 31, 1990

FINAL REPORT

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INTERNAL BIOCORROSION OF STEEL PIPES

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TABLE OF CONTENTS

PAGE #

LIST OF TABLES v

LIST OF FIGURES vi

EXECUTIVE SUMMARY viii

1.0 OBJECTIVES 1

2.0 INTRODUCTION 1

3.0 METHODS 1

3.1 Field Test Facility 2
3.2 Sample Collection and Processing 2
3.3 Analytical Methodology 3

3.3.1 Enumeration of sulfate-reducing bacteria 3
3.3.2 Radiolabeled sulfate-reduction assay 3
3.3.3 Enumeration of acid producing bacteria 3

3.4 Corrosion Rate Measurements 3
3.5 Scanning Electron Microscopy 4

4.0 RESULTS 4

4.1 First Experimental Run 4
4.2 Second Experimental Run 5
4.3 Corrosion Rates 6
4.4 Biofilm Structure and Corrosion Deposit Composition 7
4.5 Corrosion Site Morphology 8

5.0 DISCUSSION 8

5.1 Biofilm Formation 8
5.2 Effect of Biocide Treatment 9
5.3 Corrosion Rates 10
5.4 Biofilm Structure and Corrosion Deposit Composition 12
5.5 Corrosion Site Morphology 13

6.0 CONCLUSIONS 14

7.0 REFERENCES 15

8.0 TABLES 18

9.0 FIGURES 22

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TABLE OF CONTENTS continued...

LIST OF TABLES

1. Biocide injection concentrations and times used in the first and second experimental runs

2. Mean values of viable counts for sulfate-reducing and acid-producing bacteria after four weeks of colonization at the field test facility

3. Corrosion rates measured at week four prior to biocide treatment in the first and second experimental runs

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TABLE OF CONTENTS continued...

LIST OF FIGURES

1. Viable sulfate-reducing bacterial counts on sample coupons before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

2. Viable acid-producing bacterial counts on sample coupons before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

3. Turnover rates of ^{35}S-labelled sulfate to sulfide before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

4. Viable sulfate-reducing bacterial counts on sample coupons before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

5. Viable acid-producing bacterial counts on sample coupons before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

6. Turnover rates of ^{35}S-labelled sulfate to sulfide before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

7. Enhancement of general corrosion rates after biocide treatment in the first experimental run

8. Enhancement of pitting tendency rates after biocide treatment in the first experimental run

9. Enhancement of general corrosion rates after biocide treatment in the second experimental run

10. Enhancement of pitting tendency rates after biocide treatment in the second experimental run

11. Scatter plot of general corrosion rates and viable sulfate-reducing bacteria counts

12. Scatter plot of general corrosion rates and viable acid-producing bacteria counts

13. Scatter plot of pitting tendency rates and viable sulfate-reducing bacteria counts

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14. Scatter plot of pitting tendency rates and viable acid-producing bacteria counts

15. Scatter plot of general corrosion rates and turnover rates of ^{35}S-labelled sulfate to sulfide

16. Scatter plot of pitting tendency rates and turnover rates of ^{35}S-labelled sulfate to sulfide

17. Scanning electron micrograph of surface on an uncoated sample coupon from the field test facility

18. Energy dispersive X-ray spectrum from a thin corrosion deposit on the surface of a sample coupon from the field test facility

19. Energy dispersive X-ray spectrum from a thick corrosion deposit on the surface of a sample coupon from the field test facility

20. Scanning electron micrograph of surface on a coated sample coupon from the field test facility showing two distinct textural regions on thick corrosion deposits

21. Scanning electron micrograph of bacteria associated with porous regions on large corrosion deposits, (A) vibroid bacteria and (B) filamentous bacteria

22. Scanning electron micrograph of bacterial cells in successive stages of mineralization by iron sulfides, (A) cell surface deposits and (B) burial of bacterial cells (arrows).

23. Scanning electron micrograph showing incipient formation of small holes or tunnels (arrows) oriented in the direction of rolling on the surface of a sample coupon from the field test facility, (A) uncleaned surface, (B) cleaned surface and (C) nascent tunnels

24. Scanning electron micrograph showing incipient formation of small holes or tunnels (arrows) oriented in the direction of rolling on the surface of a sample coupon from the field test facility, (A) uncleaned surface, (B) cleaned surface and (C) nascent tunnels

25. Scanning electron micrograph showing area on the surface of a sample coupon devoid of corrosion deposit, (A) before cleaning, (B) after cleaning and (C) at a higher magnification after cleaning

26. Scanning electron micrograph showing tunneling corrosion on a sample coupon previously exposed to a pure culture of *Desulfovibrio vulgaris* NCIB 8303, (A) at low magnification and (B) at a higher magnification

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EXECUTIVE SUMMARY

A study was conducted to evaluate the effects of biocide dose on the development of corrosive microbial biofilms, corrosion rates, and *deposition* of *corrosion deposits* at an oilfield water injection field test facility near Wainwright, Alberta. Attached microbial populations were allowed to develop in five test lines during the first four weeks of operation during two separate experimental runs. Then biocide was injected for ten weeks into four lines in paired combinations of two different concentrations and time intervals. A different cocodiamine-based biocide formulation was used in each experimental run. In both cases, the remaining test line was used as an untreated control. Ultraviolet irradiation was used to disinfect input water streams on two test lines under biocide treatment during the last four weeks of the first experimental run.

In the first experimental run all test lines were heavily colonized by sulfate-reducing bacteria (SRB) and acid-producing bacteria (APB). Bacterial counts were lower over the corresponding period in the second experimental run, suggesting that a decrease in nutrient availability had occurred. In both experiments, a steady decline in bacterial counts was observed after biocide treatment started. Turnover rates of sulfate to hydrogen sulfide were also inhibited by biocide. Changes in dosage levels and UV disinfection of input water streams did not significantly alter biocide performance in either of the experimental runs; however, the biocide used in the second experiment was less effective against SRB than the formulation used in the first experiment. This difference, and the lack of response to changes in dosage levels, imply that biocide efficacy depends strongly on penetration through biofilms of attached bacteria. Biocide treatment did prevent further increases in corrosion rates, as observed during both experimental runs in untreated control lines; however, there were no obvious correlations between the microbiological data and corrosion rates.

Direct examination of sample coupons from the field test facility by scanning electron microscopy (SEM) revealed a variety of bacteria encrusted in successive stages of mineralization by iron sulfide corrosion deposits, as determined by energy dispersive X-ray spectroscopy. The low iron to sulfur ratio sulfides associated with the bacteria are known to be highly aggressive towards steel, but tend to be easily polarized by cathodic hydrogen. This favors the growth of hydrogen-consuming bacteria which can sustain high corrosion rates by cathodic depolarization.

Tunneling corrosion oriented in the direction of rolling was observed using the SEM in areas formerly under corrosion deposits on cleaned sample coupons from the field test facility. Similarly, tunnels were found on steel coupons previously exposed to a pure culture of *Desulfovibrio vulgaris*

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NCIB 8303. This type of corrosion damage has been attributed to organic acid production by bacteria, and appears to be a distinctive metallurgical signature for microbial corrosion.

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SOMMAIRE EXECUTIF

Une etude est conduite a fin d'evaiuer les effets qu'une dosage des biocides peut avoir sur le development des film cellulaires corrodants, le taux de corrosion, et la deposition des depots corrodants dans le systeme d'injection d'eau au centre d'essai pres de Wainwright, Alberta. Les bacteries etaient laissees se developer dans les lignes d'essai pendant les premieres quatre semaine d'operation au cours de deux essais independants. Puis les biocides etaient injectees pour dix semaines dans quatre lignes en pair de deux concentrations et intervales differentes. Une differente biocide base a cocodiamine etait utilisee dans chacun des essais. Pour tous les deux cas, la derniere ligne d'essais etait utilisee comme une ligne temoin de controle non-traitee. Rayonnment ultraviolet etait utilise a fin de desinfecter l'eau d'arrivee dans deux lignes d'essai sous traitement des biocides pendant les quatre dernieres semaines du premier essai.

Dans le premier essai, toutes les lignes d'essai montraient une concentration haute en bacteries qui reduisent les sulfates (BRS) et les bacteries qui produisent de l'acide (BPA). La concentration en bacteries etait basse pour la periode correspondante dans le deuxieme essai. Cela suggere qu'une reduction en disponibilite des nutrient est en place. Dans tous les deux essais, un declin continuel en concentration des bacteries etait observe apres avoir commence le traitement avec les biocides. Le taux de conversion des sulfates en sulfide d'hydrogene etaient reduit par les biocides. Le changement au niveau de dosage et la desinfection de l'eau d'entree par le rayonnemnet ultraviolet n'avaient pas tellement d'effet sur la performance des biocides pendant tous les deux essais. Quand meme, les biocides utilisees dans le deuxieme essai etaient moins efficaces contre les BRS que le systeme utilise pendant le premier essai. Cette difference, et la manque de reponse aux changements en niveaux de dosage, indiquent que l'efficacite des biocides depend largement sur la penetration a travers des films cellulaires des bacteries attachees. Le traitement avec les biocides avait reduit le taux de corrosion. Cela etait evident quand on comparaient les deux lignes avec la ligne temoin de controle. Pourtant, il n'y avait aucune correlation entre les donnees microbiales et le taux de corrosion.

Une inspection sous SEM des echantillons du champs montre qu'une variete des bacteries encadrait en etages de mineralisation successive qui etait determine par l'energie dispersive de rayonnment X. Les sulfide a proportion basse de fer a sulfur sont connus pour attaquer les acier mais aussi facilement polarises par les hydogene cathodes. Cela augmente le development des bacteries qui consument les hydrogenes et qui sont aussi resistante a un taux de corrosion tres eleve.

Une corrosion avec troux etait observe par SEM en direction de roulement de l'acier sur les echantillons propres laisses dans un endroit expose de corrosion. De meme, les troux de corrosion etaient observes sur les echantillons d'acier qui etaient exposes a une culture de *Desulfovibrio vulgaris* NCIB 8303. Ce genre de corrosion est attribue a l'acide organique produite par les bacteries et correspond a la corrosion microbiale.

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1.0 OBJECTIVES

Evaluate effects of biocide dose (i.e., concentration and exposure time) on microbial growth and population distributions on carbon steel coupons in the field with and without an input of viable planktonic bacteria.

Monitor biofilm structure, deposition of corrosion products, corrosion rates and corrosion site morphology under various biocide treatment protocols, and attempt to correlate *in situ* corrosion rates with microbiological data.

2.0 INTRODUCTION

Microbial biofilms are known to cause extensive fouling and corrosion of internal pipe surfaces in oil field water injection systems (Ruseska *et al.*, 1982; Chen and Chen, 1984); however, little is known about the relationships between these adherent microbial populations and corrosion rates (Hamilton, 1985). Control of corrosive bacterial activities is a serious problem and usually involves utilization of chemical biocides (Ruseska *et al.*, 1982; Maxwell *et al.*, 1987). The development of effective treatment and risk assessment protocols therefore require some knowledge of how attached microorganisms respond to biocides.

The aim of this investigation is to assess the impact of biocide dose (i.e., concentration and exposure time) on (i) kill of attached microbial populations on steel surfaces, (ii) distribution of microorganisms, (iii) microbial activity, (iv) biofilm structure, (v) deposition of corrosion products, (vi) corrosion site morphology, and (vii) corrosion rates. Any correlation between microbiological data and corrosion rates would enable prediction of risk and provide an algorithm for determining the economic benefit of any biocide treatment protocol. This is not presently possible in any field case.

3.0 METHODS

3.1 Field Test Facility

A field test facility was constructed by NOVA HUSKY RESEARCH CORPORATION (NHRC) at Unit # 13 waterplant of HUSKY OIL Ltd. Wainwright oil field in Alberta. This facility permits the growth

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and monitoring of natural microbial biofilms on five separate slip-streams. Each line can be equipped with 40 removable sample coupons, and has an individual injector system which facilitates testing of biofilm responses to various chemical compounds (e.g., biocides or corrosion inhibitors).

For this investigation, 0.95 mm diameter carbon steel coupons were used for routine analyses. In addition, the field test facility was equipped with potentiodyne probes to measure corrosion rates. An ultraviolet (UV) disinfection unit was also installed on two separate lines to kill viable planktonic bacteria before they flowed into the sample coupon legs.

The field test facility was used for two separate experimental runs. In each case, untreated water from injection system was allowed to run through the five separate test lines for four weeks. This colonization period was used to monitor biofilm growth and develop attached populations of microorganisms on the removable sample coupon surfaces. Treatment with cocodiamine biocides started at week 4 of both experimental runs and continued uninterrupted for an additional ten weeks. UV disinfection of input streams was used over the last four weeks of the first experiment. Biocide was injected into four test lines at two different concentrations and times (see Table 1 for details). The remaining test line was used as an untreated control.

3.2 Sample Collection and Processing

Carbon steel sample coupons were pulled biweekly from each line in paired sets. After removal, all sample coupons were immediately placed into sterile plastic vials which were subsequently filled with untreated water from the injection system. The specimen vials were then shipped to Calgary by plane for processing at NHRC. At NHRC, all work was done in a FORMA Scientific anaerobic chamber.

The exposed surface of each sample coupon was scraped with a sterile scalpel to remove attached microorganisms, and the residue was placed into a tube containing 10 mL of sterile Postgate B minimal salts medium supplemented with 1.0 % (w/v) NaCl (Postgate, 1984). The tubes were then sonicated for 5 minutes using a Bransonic 32 sonicator to disrupt aggregates of bacterial cells. These sample suspensions were used for all subsequent analyses.

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3.3 Analytical Methodology

3.3.1 Enumeration of sulfate reducing bacteria

Sulfate reducing bacteria (SRB) were enumerated by the Most Probable Number (MPN) technique. Serial dilutions from 10^{-1} to 10^{-8} were prepared by adding 0.5 mL of the sample suspensions or water to tubes containing 4.5 mL of Postgate B medium supplemented with 1.0 % (w/v) NaCl (Postgate, 1984). The tubes were then incubated at 25 °C for up to 21 days. Blackening to a tube due to iron sulfide formation was a positive indication of growth by sulfate reducing bacteria. Standard probability tables were then used to express the number of positive tubes as viable SRB per cm^2 of sample coupon surface.

3.3.2 Radiolabeled sulfate reduction assay

The activity of SRB was assessed by adding 1.0 mL aliquots of sample suspensions to tubes containing 1.0 mL of Postgate C medium supplemented with 1.0 % (w/v) NaCl and 0.8 μCi/mL of ^{35}S-sulfate. For one series of determinations, nitrate or methanol was added to final concentrations of 60 mM and 0.02 % (v/v), respectively. A cup containing a paper wick saturated with 0.5 mL of 1.5 M zinc acetate was suspended from rubber stoppers in each tube to trap evolved H_2S. The assays were terminated after 6 hours of incubation at 25 °C by adding 1.0 mL of 6 M HCl to each tube. After an additional 24 hours, the wick cup assemblies were removed and placed into 10 mL of scintillation fluid. These samples were then counted a day later using a Rack Beta Model 1211 scintillation counter, and the μmoles H_2S produced.cm^{-2}.day^{-1} calculated.

3.3.3 Enumeration of acid producing bacteria

Acid producing bacteria (APB) were enumerated by serial dilution of sample suspensions and water from 10^{-1} to 10^{-7} in BTI/APB medium (Bioindustrial Technologies Inc., Grafton, New York, U.S.A.). The growth of APB was revealed by a change in culture medium colour from red to yellow; the number of APB in the original samples was then calculated from the highest dilution at which growth occurred using standard probability tables.

3.4 Corrosion Rate Measurements

Potentiodynaic polarization measurements were done at 3 to 4 week intervals using a Petrolite model M-4100 potentiodyne analyzer. General corrosion rates were determined from current

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density values at the intersection point of cathodic Tafel slopes and open circuit potentials. Pitting tendency rates were estimated from current density values at the intersection of cathodic Tafel slopes and return anodic curves.

3.5 Scanning Electron Microscopy

Sample coupons were taken from each test line at the conclusion of both experimental runs for scanning electron microscope (SEM) examination. Immediately after removal, the coupons were immersed in vials containing 2.0 % (v/v) glutaraldehyde fixative in untreated water from the injection system. After 24 hours, the coupons were washed 3 to 5 times with 0.1 M cacodyllic buffer at pH 7.0 and dehydrated through an ethanol-freon series to 100 percent freon. The sample coupons were then allowed to air dry. Cleaned carbon steel coupons previously exposed to *Desulfovibrio vulgaris* NCIB 8303 were obtained for examination from Dr. T. Jack (NOVA HUSKY RESEARCH CORPORATION). Specimens were examined before and after gold sputter coating using a Hitachi S-2500 SEM operating at 15 kV. The SEM was equipped with a Tracor Northern energy dispersive X-ray spectrometer. Energy dispersive X-ray spectroscopy (EDS) was conducted in the selected area electron beam raster mode, and spectra were obtained by collecting counts for up to 200 seconds (live time).

4.0 RESULTS

4.1 First Experimental Run

In the four weeks prior to biocide treatment, all test lines were colonized to > 10^8 SRB per cm^2 (Figure 1). The APB counts ranged from 10^5 to 10^8 per cm^2 (Figure 2). These numbers are higher than those obtained during previous work at the field test facility (see Table 2 for a comparison of data). The reasons for this are not entirely clear; however, some seasonal variability in size of attached bacterial populations might be expected as ambient temperatures and injection water chemistry (i.e., nutrient availability for bacterial growth) change.

After biocide injection started at week four, the numbers of attached APB decreased rapidly (> 99.9 % kill within four weeks), whereas the SRB counts declined slowly (Figures 1 and 2). Although no major differences were detected between the various biocide treatment protocols, a ten week period of sustained treatment was needed to reduce the SRB counts to steady state levels near 10^4 cells per cm^2, as observed in previous studies with this biocide (Ferris, 1989a). Disinfection of input water by UV irradiation did not significantly enhance biocide performance.

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Biocide treatment effectively inhibited sulfate-reduction activity (Figure 3). Again, no differences were observed between the various treatment protocols. This suggests that enough biocide penetrated through the bacterial biofilms to control production of hydrogen sulfide, even at the reduced injection concentrations.

Results from the control line were extremely unusual. Counts for SRB and APB, and sulfate-reduction activity decreased dramatically between weeks four to eight, then increased slowly thereafter (Figures 1,2 and 3). There is no good explanation for this data; however, a similar trend was observed in the last study at the field test facility (Ferris, 1989a). These abnormalities might be related to variations in injection water chemistry. Further investigations are needed to determine the source of this problem, and assess implications for biocide performance.

4.2 Second Experimental Run

All test lines were colonized to > 10^7 SRB per cm^2 in the four weeks prior to biocide treatment (Figure 4). The APB counts ranged from approximately 10^3 to 10^5 per cm^2 (Figure 5). These numbers are lower than those obtained during the first experimental run (Table 2), as might be expected for seasonal changes in ambient temperature or injection water chemistry.

The numbers of attached SRB and APB declined slowly after biocide injection started at week four (Figures 4 and 5). There were no consistent differences between the various treatment protocols; however, the performance of the 20 % active ingredient (A.I.) biocide against SRB was poor compared to that obtained with the 35 % A.I. formulation used in the first experimental run. Viable SRB counts were reduced only by two orders of magnitude to approximately 10^6 per cm^2 with the 20 % A.I. biocide (ca. 99.00 % kill), whereas the 35 % A.I. biocide induced a 99.99 % kill to near 10^4 SRB per cm^2. This implies that the 35 % A.I. biocide is a more effective formulation than the 20 % A.I. biocide.

Inhibition of hydrogen sulfide production by SRB was sporadic as intermittent bursts of activity occurred during biocide treatment (Figure 6). Apart from these periodic episodes of increased activity, there were no differences between the various treatment protocols; however, the levels of sulfate-reduction activity prior to biocide treatment were lower than those determined in the first experimental run. This data and the viable bacterial counts provide additional evidence which suggests that environmental parameters, such as temperature or injection water chemistry, strongly influence the growth and activity of bacteria on internal pipe surfaces.

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The unusual fluctuations in bacterial numbers and activity on samples from the control line continued during the second experimental run. Counts for SRB and APB, and sulfate-reduction activity decreased dramatically after week four and bounced back at week 8. This cycle was repeated over the last 6 weeks.

4.3 Corrosion Rates

Corrosion rate measurements from each test line varied considerably before biocide treatment in both experimental runs (Table 3). These variations can be attributed to turbulent flow conditions which can give rise to cavitation events and gas break-out around corrosion probe electrodes. These phenomena effectively perturb electrical potential differences between electrodes and confound rigorous determinations of instantaneous corrosion rates from current densities.

The mean corrosion rate values determined from week four data of the second experimental run were approximately 2 to 3 times higher than those of the first experimental run (Table 3). Conversely, viable counts for SRB and APB, and sulfate- reduction activity were lower at week four in the second experiment (Figures 1 and 4). These results provide evidence which suggests that injection water chemistry changed between experiments and became more corrosive.

Differences in absolute corrosion rate values (see Table 3) made it difficult to directly compare effects of the various biocide treatment protocols. Consequently, corrosion rates measured in each test line after the start of biocide injection were normalized by corresponding week four values to obtain proportional ratios. These ratios indicate how corrosion rates changed during biocide treatment.

In the first experimental run, general corrosion rates increased more than 40 times in the control line during the corresponding ten week period of biocide treatment (Figure 7). Similarly, control line pitting tendency rates were more than 150 times higher at week fourteen (Figure 8). On the other hand, biocide effectively prevented a gross increase of corrosion rates in the test lines. Apart from an initial increase of 10 to 15 times in general corrosion rates with higher biocide concentrations, there were no significant differences between the various treatment protocols.

During the second experimental run, control line general corrosion and pitting tendency rates increased only 10 times by week fourteen (Figures 9 and 10). At the higher biocide concentration in lines 2 and 4, there was no increase in the corrosion rates; however, the general corrosion and

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pitting tendency rates increased 20 to 150 times in lines treated with the lower concentration of biocide. The implication of this difference is that corrosion rates may actually be accelerated by low concentrations of certain biocides, perhaps through chemical reactions with dissolved constituents in the injection water.

Scatter plots of general corrosion rates versus viable SRB and APB counts are presented in Figures 11 and 12. In each case, a slight trend towards higher corrosion rates with increasing numbers of bacteria is evident; however, direct mathematical relationships between the data are not obvious. The same observations apply to scatter plots of pitting tendency rates as a function of bacterial counts (Figures 13 and 14).

There were no distinct trends evident in scatter plots of general corrosion and pitting tendency rates versus SRB activity (Figures 15 and 16); however, some of the higher corrosion rates were associated with low levels of hydrogen sulfide production. This suggests that SRB active in the laboratory assay medium are not particularly aggressive in the field.

4.4 Biofilm Structure and Corrosion Deposit Composition

Direct examinations of sample coupons prepared for SEM revealed an uneven distribution of corrosion deposits on the exposed steel surfaces (Figure 17). Typically, most of the surface area on a sample coupon was covered by a thin corrosion deposit layer which generated Fe and S peaks in corresponding EDS spectra (Figure 18). Thicker deposits with a caked appearance occurred at random on the coupon surfaces. The thick corrosion deposits on uncoated specimens were prone to charging in the electron beam (Figure 17), and invariably contained more S in relation to Fe than the thin deposits (Figure 19). There were no differences in the morphology or composition of the corrosion deposits among the various biocide treatment protocols.

At higher magnifications, two texturally distinct regions were evident on the thicker corrosion deposits (Figure 20). The bulk of these deposits appeared to be relatively smooth, whereas peripheral areas often exhibited a porous sponge-like texture. The elemental composition of these different textural regions was the same; however, bacterial cells were abundant and easily discerned in the porous regions (Figure 21). In contrast, bacteria were not found in areas of the thick corrosion deposit with a smooth appearance. The thinner deposits covering the surface of most sample coupons were similarly smooth and devoid of bacteria.

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Bacteria prominent in the porous regions of the thick corrosion deposits were morphologically diverse, as expected for a complex biofilm community. Bacterial cells with a curved shape characteristic of *Desulfovibrio* species were commonly observed (Figure 21 A). Elongated filamentous forms were also found, but their identity is not known (Figure 21B). Biocide treatment had no obvious affects on the morphology or distribution of bacteria.

Closer examination of some vibroid bacteria revealed cells in successive stages of mineralization by iron sulfides (Figure 22A). In some cases, it was possible to discern outlines of bacterial cells buried in the iron sulfide matrix of the corrosion deposit (Figure 22B).

4.5 Corrosion Site Morphology

There was no obvious pitting of steel surfaces in areas formerly under corrosion deposits on cleaned sample coupons examined by SEM; however, incipient formation of small holes or tunnels in the direction of rolling (i.e., perpendicular to the coupon surface) was evident, particularly in machined grooves on the coupon surface (Figures 23 and 24). This tunneling was not observed in areas devoid of corrosion deposit (compare Figure 25 with Figures 23 and 24).

Tunneling corrosion in the direction of rolling was also observed on cleaned sample coupons cut from steel line pipe which were previously exposed to *Desulfovibrio vulgaris* NCIB 8303. The tunneling was most pronounced in well developed corrosion pits, and appeared at higher magnifications as a form of intergranular attack (Figure 26). Incipient tunnel formation comparable to that observed on the sample coupons from the field test facility was evident in smaller corrosion pits.

5.0 DISCUSSION

5.1 Biofilm Formation

Surface colonization has been described as a basic bacterial growth strategy in nature (Costerton, *et al.*, 1978). Many examples have been reported since Zobell described the attachment and growth of marine bacteria on glass slides (Zobell and Allen, 1933, Zobell, 1943). The kinetics of bacterial surface colonization are complex (Caldwell *et al.*, 1981; Lawrence *et al.*, 1987); however, the process depends fundamentally on a combination of adhesion by means of extracellular polymers and sustained growth of attached bacteria. The net result is formation of microcolonies

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which eventually coalesce to form complex biofilm communities similar to those occurring on the sample coupons from the field test facility, and on internal pipe surfaces in other water injection systems.

Nutritient availability plays an important role in bacterial surface colonization. Under starvation conditions, the adhesiveness of bacterial cells tends to be reduced and surface colonization rates decrease (Salas and Geesey, 1983). Furthermore, sloughing of cells from established biofilms commonly occurs in response to nutrient depletion (Delaquis *et al.*, 1989; Characklis and Cooksey, 1983). These observations suggest that a poorer supply of nutrients contributed to lower sulfate-reduction activity levels and viable bacterial counts in the second experimental run. The reason(s) for such a change in nutrient availability are not entirely clear; however, injection water chemistry can be highly variable with respect to both inorganic and organic consitutents (Ostroff, 1979). Additional studies are needed to assess in greater detail how water chemistry influences bacterial surface colonization in oilfield water injection systems.

5.2 Effect of Biocide Treatment

Models commonly used to describe the inactivation of bacteria by bactericidal agents predict that viability should decrease exponentially with time (LeChevallier *et al.*, 1988). An important assumption for applications of such models to attached bacterial populations is that surface recolonization by viable planktonic (i.e., free-living) bacteria must be negligible. When this assumption is invalid, rapid attachment of planktonic cells would be expected to sustain biofilm growth (Caldwell *et al.*, 1981), and reduce biocide efficacy. The near exponential decrease in viable bacterial counts induced by biocide treatment in both experimental runs is therefore indicative of low attachment rates of free-living cells. This provides a good explanation for why UV disinfection of input water streams did not enhance biocide performance during the first experimental run.

The bactericidal action of biocides depends not only on concentration, but also on contact time (LeChevallier *et al.*, 1988); however, reducing biocide injection concentrations and exposure times by up to 50 percent did not significantly alter performance in the two experimental runs. This suggests inactivation of attached corrosion enhancing bacteria on internal pipe surfaces is not solely related to the dosage level of a biocide. The implication is that when biocide is used at bactericidal levels, penetration into a biofilm (i.e., mass transfer) is an important rate-limiting step (LeChevallier *et al.*, 1988). Additional evidence in support of this concept comes from the rapid inactivation of APB in comparison to SRB, as noted particularly in the first experimental run. In

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this context, the increased sensitivity of APB suggests these bacteria reside on the outside of biofilms while SRB are probably buried and less accessible to biocide.

Selective penetration into bacterial biofilms has been proposed as an explanation for differences in performance between chemicals used as disinfectants in municipal water distribution systems (LeChevallier *et al.*, 1988). Similar arguments have been advanced to explain why some biocides that are effective against planktonic bacteria have very little effect on attached bacterial populations (Ruseska *et al.*, 1982). The inference is biocide efficacy depends strongly on diffusion rate through a biofilm. For this reason, the poorer performance of the 20 % A.I. biocide in comparison to the 35 % A.I. biocide is probably a function of slower rates of diffusion. Although both biocide formulations contained the same active ingredient, cocodiamine contents varied with the 20 % A.I. formulation containing more diluent than the 35 % A.I. product. Presumably, the chemicals used as diluents in the 35 % A.I. biocide promoted the development of conditions which were more favorable for the diffusion of bactericidal cocodiamine through the biofilms. Further research is needed to determine how important chemical diluents are for biocide efficacy.

The unusual fluctuations of bacterial counts and sulfate-reduction activity in the control lines during both experimental runs is perplexing. There are several possible explanations for the observed variations in the data; however, the exact cause(s) are not know. A fundamental consideration to begin with is that the field test facility is run in a loop off the main water plant injection line. Chemicals used in operations upstream of the water plant, such as oxygen scavengers and scale inhibitors, are periodically flushed through the field test facility lines and may inhibit bacterial growth. Scale inhibitors are a particularly likely cause as they complex precipitate-forming multivalent metal cations which are necessary for bacterial growth (Ferris and Beveridge, 1985; Ferris, 1989b). Another possibility for the variable growth of bacteria in the control line is periodic nutrient limitation. This might stem from changes in injection water chemistry, as discussed earlier. Both of the above explanations are problematic in that bacterial counts and activity levels in test lines under biocide treatment were relatively uniform. The implication is that some chemical(s) in the biocide formulations may actually sustain the growth of attached bacteria by acting directly as nutrients and/or by forming some type of protective barrier layer.

5.3 Corrosion Rates

Electrochemical techniques of corrosion rate measurements are increasingly popular in field operations, due primarily to the rapidity with with these measurements can be made (Chamberlain, 1984). Long-term corrosion studies using wieght-loss coupons take days or weeks

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to complete, whereas an electrochemcial measurement requires at most several hours (EG&G Applied Instrument Group, 1982). The disadvantage of electrochemical corrosion rate determinations is that any perturbation of electrical potential difference and current flow between probe electrodes can cause significant deviations in measured values (Patton, 1979). For example, cavitation or gas break-out may occur around electrodes in lines under turbulent flow conditions, effectively decreasing the working area of the electrodes and surface current density. Conversely, a build-up of electrically conductive iron sulfide corrosion deposit not only increases the working area of electrodes, but also gives rise to galvanic coupling effects which can stimulate corrosion (Barnartt, 1976). The first case provides an account for the observed differences in corrosion rates between test lines during both experimental runs at the field test facility (Table 2). On the other hand, the second case explains the increased average corrosion rate values determined prior to biocide treatment during the second experiment. This corresponded with the initiation of production from a sour well upstream of the field test facility.

Normalization of corrosion rates measured after the start of biocide treatment by corresponding week four values facilitated direct comparisons of the various treatment protocols. In general, biocide treatment effectively stopped corrosion rates from increasing beyond initial levels measured at week four. At the same time, bacterial counts and sulfate-reduction activity decreased. These data provide compelling evidence which confirms that inactivation of attached bacteria on pipe surfaced by biocides can effectively maintain lower corrosion rates (Chen and Chen, 1984). The only exception was with the lower concentrations of the 20 % A.I. biocide. In this case, corrosion rates actually increased as bacterial counts and sulfate-reduction activity declined. This implies that the 20 % A.I. biocide at low concentrations was not effective against bacteria which were particularly aggressive in the field. Perhaps these bacteria were buried deep within corrosion deposits and in more intimate contact with the steel surface. Again, this reinforces the concept that penetration of bactericidal agents through biofilms is critical to performance as diffusion limitations appear to be a problem at lower biocide concentrations (LeChevallier, *et al.*, 1988; Ruseska, *et al.*, 1982).

An indefinite trend towards higher corrosion rates with increasing numbers of SRB and APB was evident in scatter plots of data aquired during these investigations; however, there was no correlation between corrosion rates and sulfate-reduction activity levels of the SRB. The lack of any direct relationship between microbiological data and corrosion rates in not unusual (Chen and Chen, 1984; Hamilton, 1985), suggesting that mechanisms of microbial corrosion are complex and coupled to other abiotic electrochemical corrosion processes.

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The distribution of data in scatter plots of corrosion rates versus sulfate-reduction activity showed that higher corrosion rates were associated with apparently dormant SRB. Such inactivity might be explained by an inability of the bacteria to use lactate, the only carbon source in the sulfate-reduction assay culture medium (Postgate, 1984). The implication is that corrosion is enhanced in the field by SRB which utilize acetate or other fatty acids instead of lactate. This is consistent with other data which suggests that most SRB in natural biofilms use hydrogen and acetate as their principal sources of energy and carbon (Hamilton, 1985).

5.4 Biofilm Structure and Corrosion Deposit Composition

Direct examination of sample coupons by scanning electron microscopy revealed a variety of bacteria, including vibroid (i.e., curved) cells reminiscent of classical sulfate-reducing *Desulfovibrio* species (Postgate, 1984). The bacterial cells were commonly found in successive stages of mineralization by amorphous iron sulfides. This appeared to eventually cause an entrainment of bacteria within surface corrosion deposits. Mineralization of bacterial cells in sedimentary environments is well documented, and occurs as a result of epitaxial nucleation and crystal growth (Ferris *et al.*, 1987; Ferris *et al.*, 1989). Anionic structural polymers in the cell walls of bacteria concentrate dissolved metal cations which subsequently precipitate at the cell surface by reacting with counterions in solution (Beveridge *et al.*, 1983; Beveridge, 1989). Further growth of these deposits can proceed via homogenous crystal nucleation reactions (Berner, 1980). These types of processes provide an explanation for the observed mineralization of bacteria on the sample coupons from the field test facility. Dissolved iron liberated from the steel surface by anodic disolution reactions was probably complexed at the surfaces of attached bacteria. Then bound iron reacted with biogenic or indigenous sulfide to produce nuclei for the growth of the amorphous iron sulfide precipitates.

The entrainment of bacterial cells within an iron sulfide corrosion deposit matrix has some important implications for mechanisms of microbial corrosion. According to the classical theory of von Wolzogen Kuhr (von Wolzogen Kuhr and van der Vlugt, 1934), hydrogen-consuming bacteria can remove cathodic hydrogen from steel (i.e., depolarize cathodic surfaces) accelerating corrosion. Attempts to demonstrate this in the laboratory with pure bacterial cultures have largely failed to mimic corrosion rates observed in the field, implying a more complex process. This is interesting as results from other studies have established that chemically synthesized or biogenically formed iron sulfides enhance corrosion rates which later tail off as the sulfide matrix becomes polarized by cathodic hydrogen (King and Wakerley, 1973; Smith and Miller, 1975); however, when hydrogen-consuming bacteria are added to systems in combination with iron

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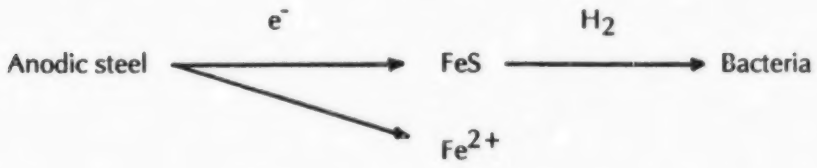
sulfides, high rates of corrosion are sustained (Jack *et al.*, 1986). These results suggest bacteria enhance corrosion by removing cathodic hydrogen from iron sulfides galvanically coupled to steel surfaces. In this context, the depolarization theory of von Wolzogen Kuhr infers a series of reactions in the presence of bacteria (King and Wakerley, 1973):



The presence of bacteria embedded directly in iron sulfide corrosion deposits as observed by SEM is consistent with this modified version of the cathodic depolarization theory for microbial corrosion.

Corrosive effects of iron sulfides commonly found in situations where corrosion occurs in petroleum production bear a direct relationship to the sulfur content of the sulfide species (Smith and Miller, 1975). In general, compounds with a low iron to sulfur ratio (e.g., pyrite, FeS_2) are more corrosive than sulfides with a high iron to sulfur ratio (e.g., mackinawite, FeS_{1-x}). The precise reasons for this are not clear; however, crystal structure defects in iron sulfides give rise to semiconductor properties which apparently make these compounds easily polarizable, particularly as sulfur content increases (King and Wakerley, 1973; Smith and Miller, 1975). This might occur through interstitial adsorption of molecular cathodic hydrogen, or by H-S bonding of cathodic atomic hydrogen within the sulfide matrix (King and Wakerley, 1973). Removal or disbonding of this polarizing hydrogen by bacteria not only sustains continued high rates of corrosion, as desribed earlier (Jack *et al.*, 1986), but also provides a source of energy for bacterial growth (Odom and Peck, 1984). For these reasons, iron sulfides with a low iron to sulfur ratio may afford conditions which are more favorable to energy coupling reactions of hydrogen-consuming bacteria. This provides a reasonable account for the preferential growth of bacteria in corrosion deposits containing a higher percentage of sulfur.

5.5 Corrosion Site Morphology

Recent microbiological studies of internal and external corrosion sites in gas pipelines showed that large numbers of organic acid-producing bacteria may be present along with or even in the absence of SRB (Pope *et al.*, 1988). Analyses of bulk corrosion deposits and fluids from these sites revealed significant concentrations of a variety of organic acids, with acetic and lactic acids

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predominant. Subsequent SEM examinations of corrosion pits in pipe walls showed that numerous tunnels had developed oriented in the direction of rolling. Similar tunneling corrosion was observed on test specimens of carbon steel immersed in deaerated 20,000 ppm acetic acid at a pH of 2. Mineral acids failed to generate tunnels, so this distinct form of corrosion was proposed as a unique form of damage caused by APB (Pope *et al.*, 1988); however, it is important to note that many species of SRB are acetogenic (Postgate, 1984; Hamilton, 1985). The tunneling effect should therefore be regarded as a distinguishing characteristic of microbial corrosion in general, and not of any specific group of bacteria. This is emphasized further by results from the present investigation. Nascent tunnel formation was observed in areas formerly under corrosion deposits on sample coupons from the field test facility where SRB were more abundant than APB. Tunneling corrosion also appeared in corrosion pits on carbon steel coupons exposed to a pure culture of *Desulfovibrio vulgaris* NCIB 8303. In view of these observations, it seems reasonable to conclude that corrosion of carbon steel due to organic acids is a viable mechanism whereby at least some microbial corrosion of steel pipe occurs. Moreover, the presence of tunneling seems to provides a useful metallurgical signature indicative of microbial corrosion.

6.0 CONCLUSIONS

- Injection water chemistry (i.e., nutrient availability) appears to play an important role in determining rates and extent of bacterial surface colonization at the field test facility. Further studies are needed to assess in greater detail how water chemistry influences the development of attached populations of corrosion enhancing bacteria in oilfield water injection systems.

- Reducing biocide injection concentrations by up to 50 percent did not adversely affect performance. Also, shortening the frequency of injection had no discernable impact on the efficacy of the biocides. Substantial savings on biocide treatment costs could be recognized from similar, carefully monitored, reductions in field operations.

- Biocide efficacy seems to be strongly dependent on rates of diffusion through bacterial biofilms. Chemicals used as diluents in biocide formulations may selectively enhance biocide penetration and performance. Additional research is needed to evaluate the importance of chemical diluents for biocide efficacy.

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- Some chemicals in biocide formulations may actually sustain the growth of attached corrosion enhancing bacteria, possibly by acting directly as nutrients and/or by forming some type of protective layer over biofilms. Chemical compatibility studies are needed to ensure that biocide formulations do not create more biofouling problems than they solve.

- At effective biofilm-penetrating concentrations, biocide treatment prevents increases in corrosion rates; however, correlations between microbiological data and corrosion rates are not readily apparent. Such correlations would be extremely valuable in predicting risk of microbial corrosion.

- The formation of iron sulfide corrosion deposits on steel surfaces contributes to microbial corrosion by coupling entrained bacteria to cathodic half-cell reactions. Removal or disbonding of cathodic hydrogen from the iron sulfide matrix by hydrogen-consuming bacteria can sustain high corrosion rates, as inferred by the classic cathodic depolarization theory of microbial corrosion.

- Bacteriogenic organic acid production contributes to at least some microbial corrosion of steel, and gives rise to tunneling oriented in the direction of rolling. This is a distinguishing characteristic of microbial corrosion in general, and not of any specific group of bacteria.

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8.0 TABLES

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TABLE 1: Biocide Injection Concentrations and Times Used in the First and Second Experimental Runs

BIOCIDE INJECTIONS SCHEDULE*				
TEST LINE	FIRST EXPERIMENT		SECOND EXPERIMENT	
	Concentration (ppm)	hours on/off	Concentration (ppm)	hours on/off
1	0 (Control)	--	0 (Control)	--
2	250	6/42	500	8/88
3	175	6/42	250	8/88
4	250	3/21	500	4/44
5	175	3/21	250	4/44

*NOTE: Cocodiamine based biocide formulations were used for both experimental runs. The product used in the first experiment contained 35% active ingredient, whereas the product used in the second experiment contained 20% active ingredient. The compound formula for cocodiamine is C_2H_{25} - $NH(CH_2)_3$ - NH_2 (Ostroff, 1979).

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TABLE 2: Mean Values of Viable Counts for Sulfate-Reducing and Acid-Producing Bacteria After Four Weeks of Colonization at the Field Test Facility

VIABLE BACTERIAL COUNTS		
EXPERIMENT	**SULFATE-REDUCING BACTERIA**	**ACID-PRODUCING BACTERIA**
Run No. 1	7.6×10^8	2.5×10^7
Run No. 2	1.1×10^8	2.5×10^3
Previous Study*	7.9×10^5	1.0×10^3

***NOTE:** from Ferris, 1989a.

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TABLE 3: Corrosion Rates Measured at Week Four (prior to biocide treatment) in First and Second Experimental Runs

CORROSION RATE (mm/y)				
TEST LINE	FIRST EXPERIMENT		SECOND EXPERIMENT	
	General	Pitting	General	Pitting
1	0.015	0.080	0.007	0.251
2	0.104	3.180	5.720	121.920
3	11.430	162.560	1.168	18.796
4	0.184	4.950	15.870	335.600
5	0.838	15.750	0.203	5.842
MEAN	2.514	37.304	4.599	96.482
STANDARD ERROR	±2.233	±31.426	±3.005	±63.773

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9.0 FIGURES

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FIGURE 1. Viable sulfate-reducing bacterial counts on sample coupons before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

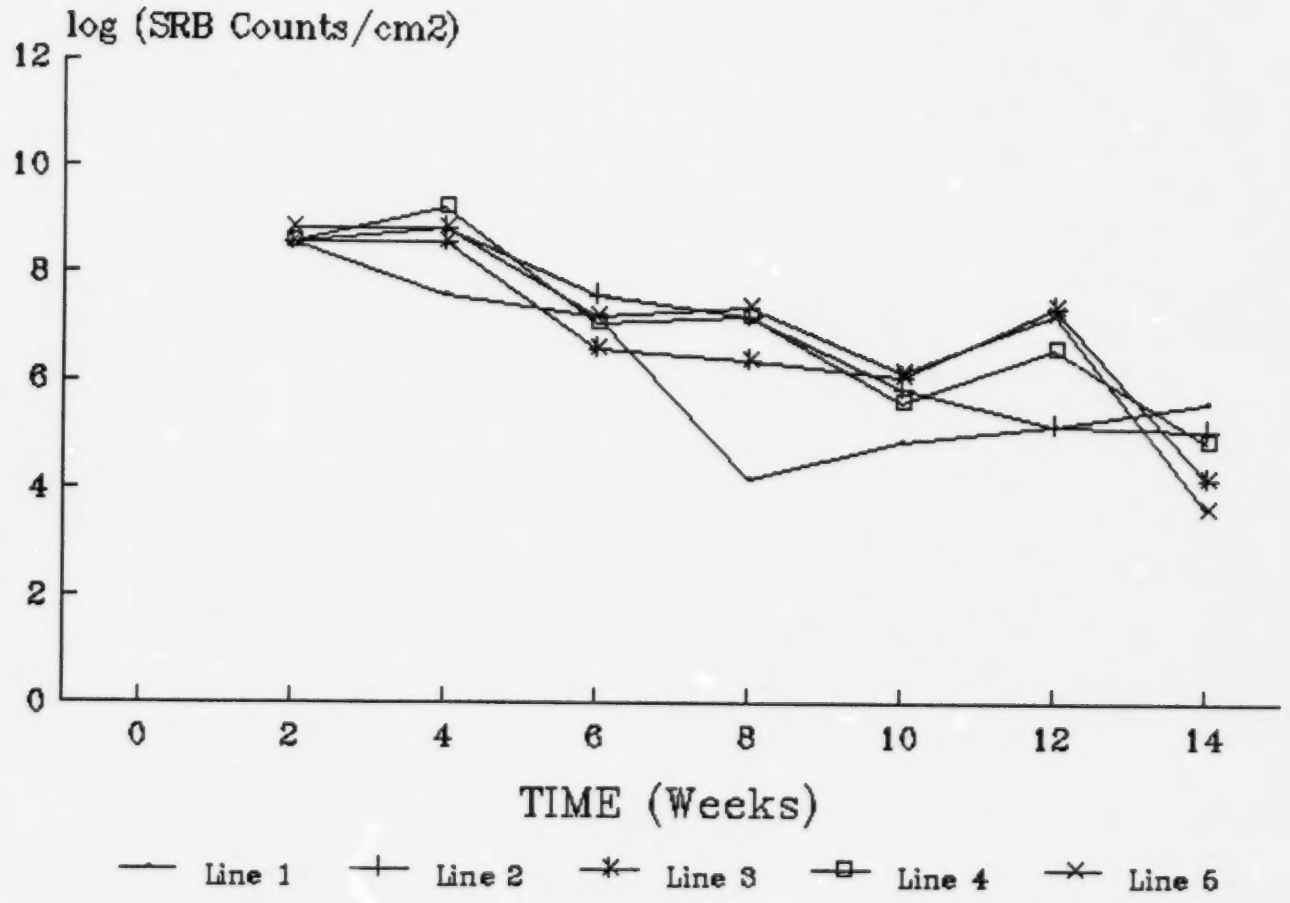

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FIGURE 2. Viable acid-producing bacterial counts on sample coupons before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

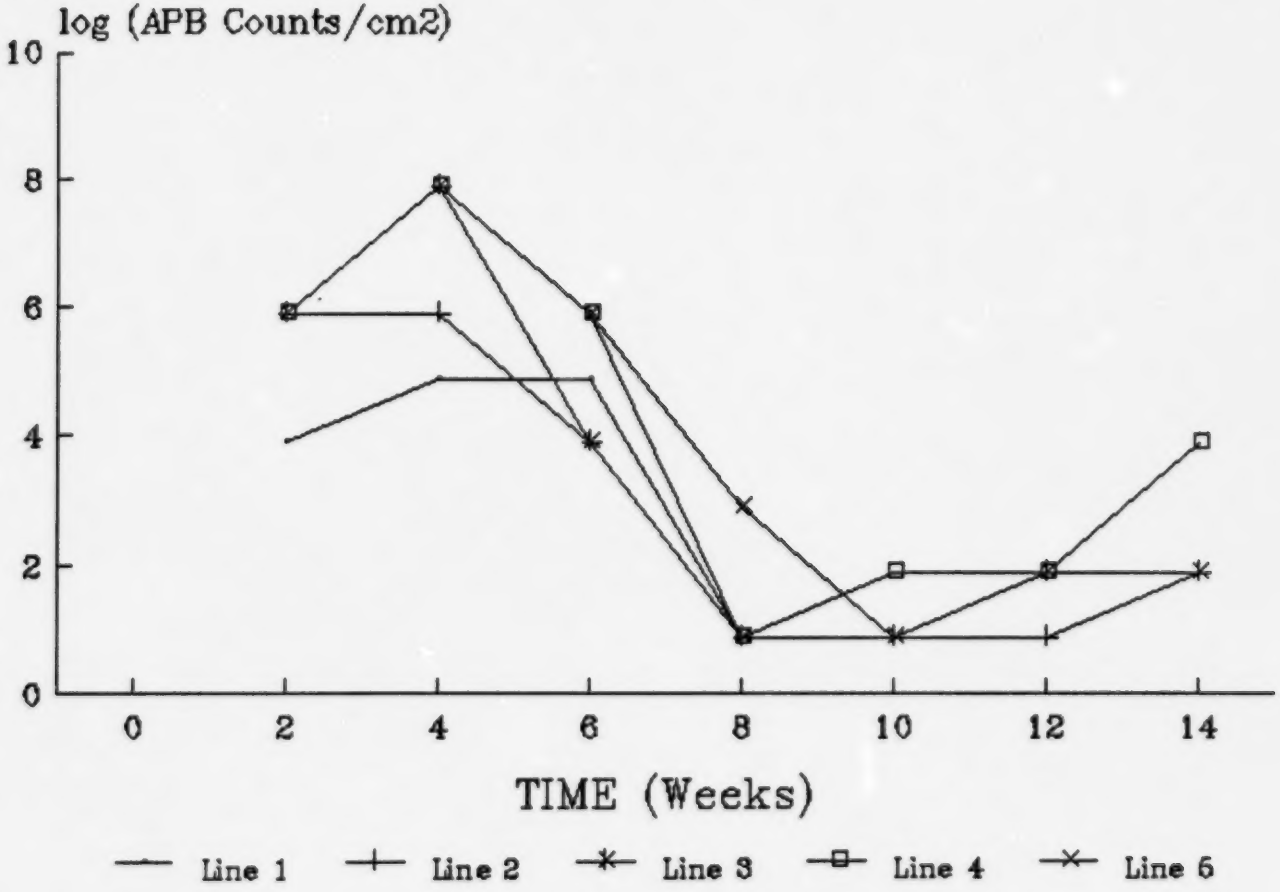

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FIGURE 3. Turnover rates of ^{35}S-labelled sulfate to sulfide before and after biocide treatment during the first experimental run. Biocide treatment started at week 4.

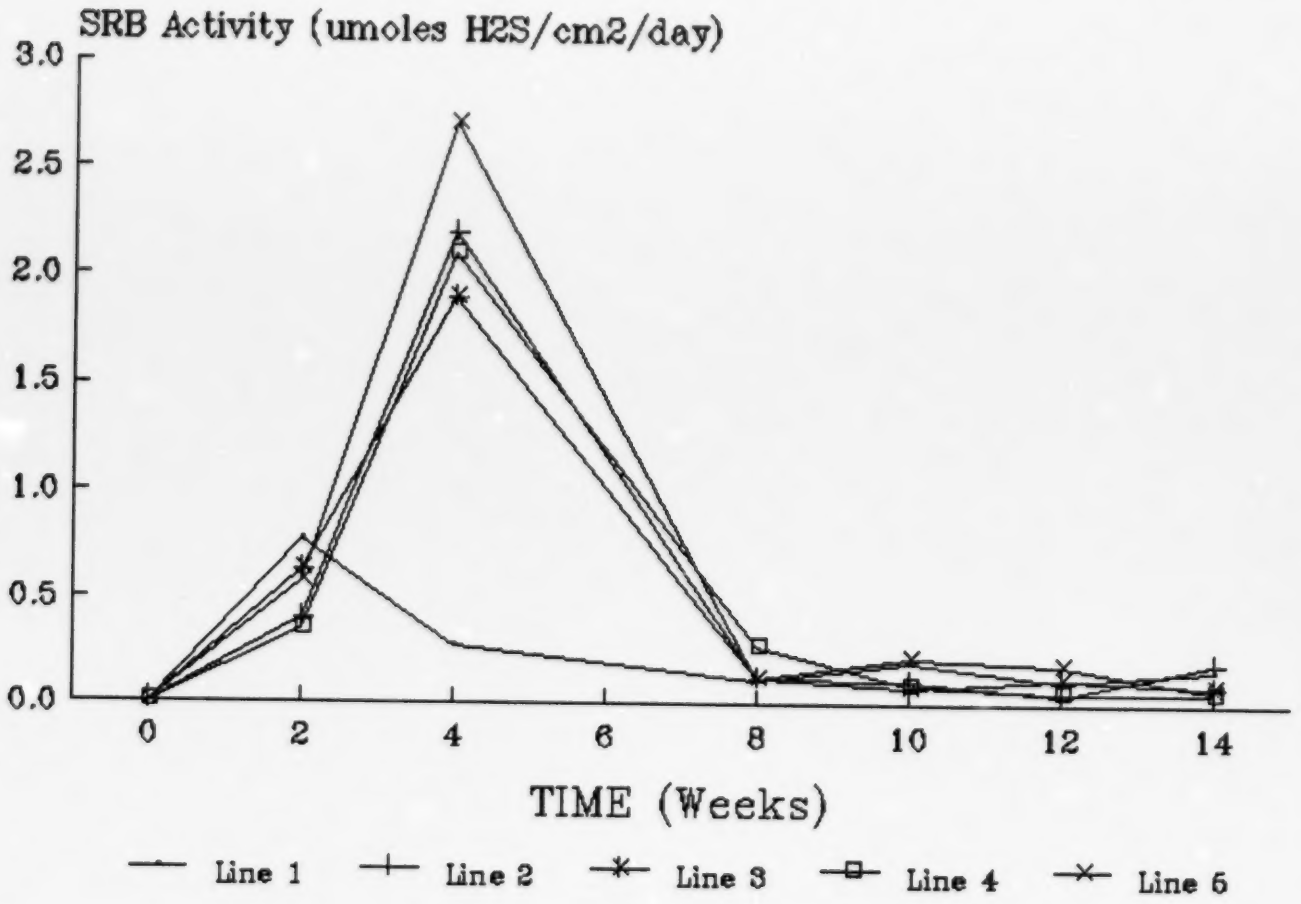

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FIGURE 4. Viable sulfate-reducing bacterial counts on sample coupons before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

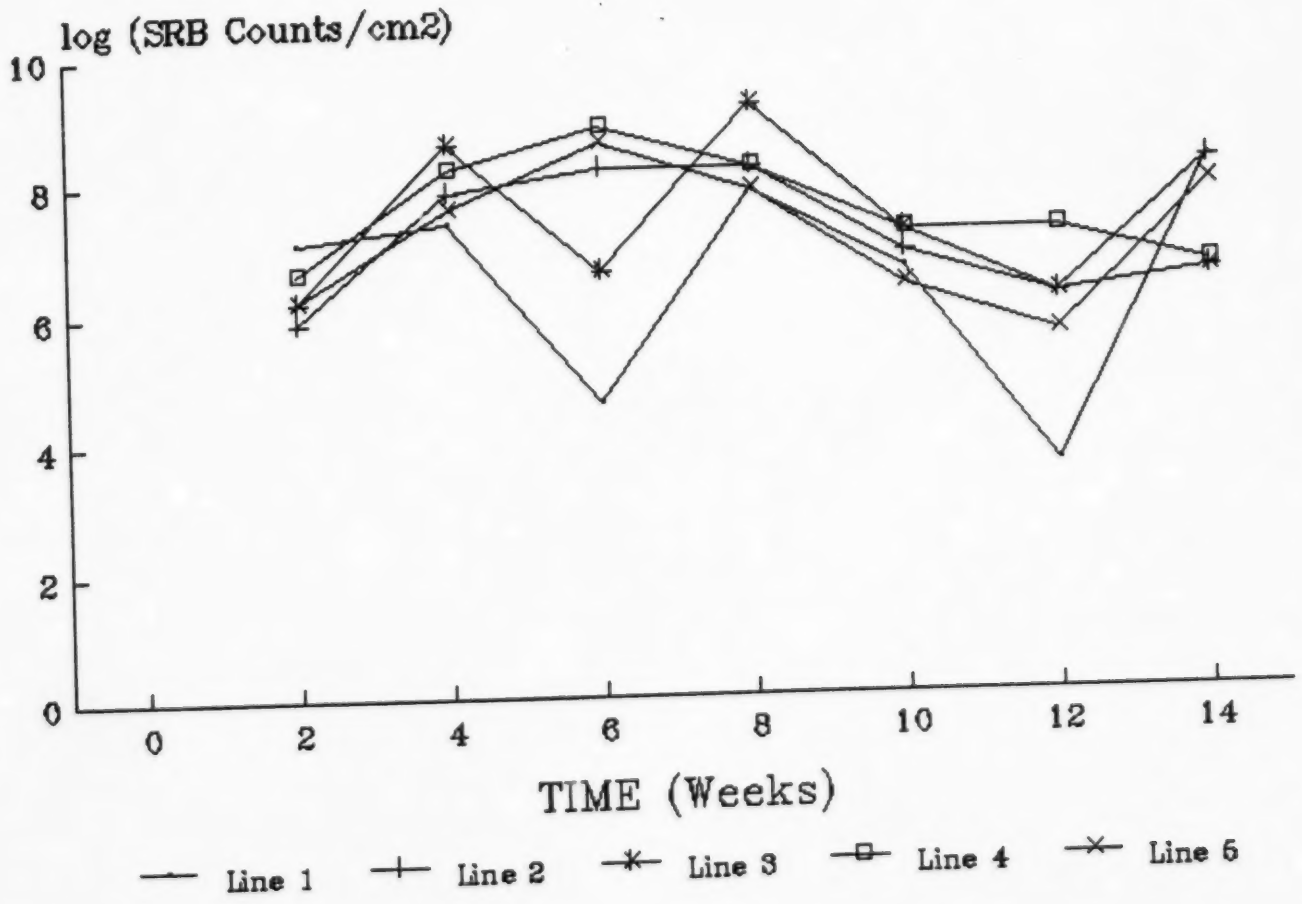

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FIGURE 5. Viable acid-producing bacterial counts on sample coupons before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

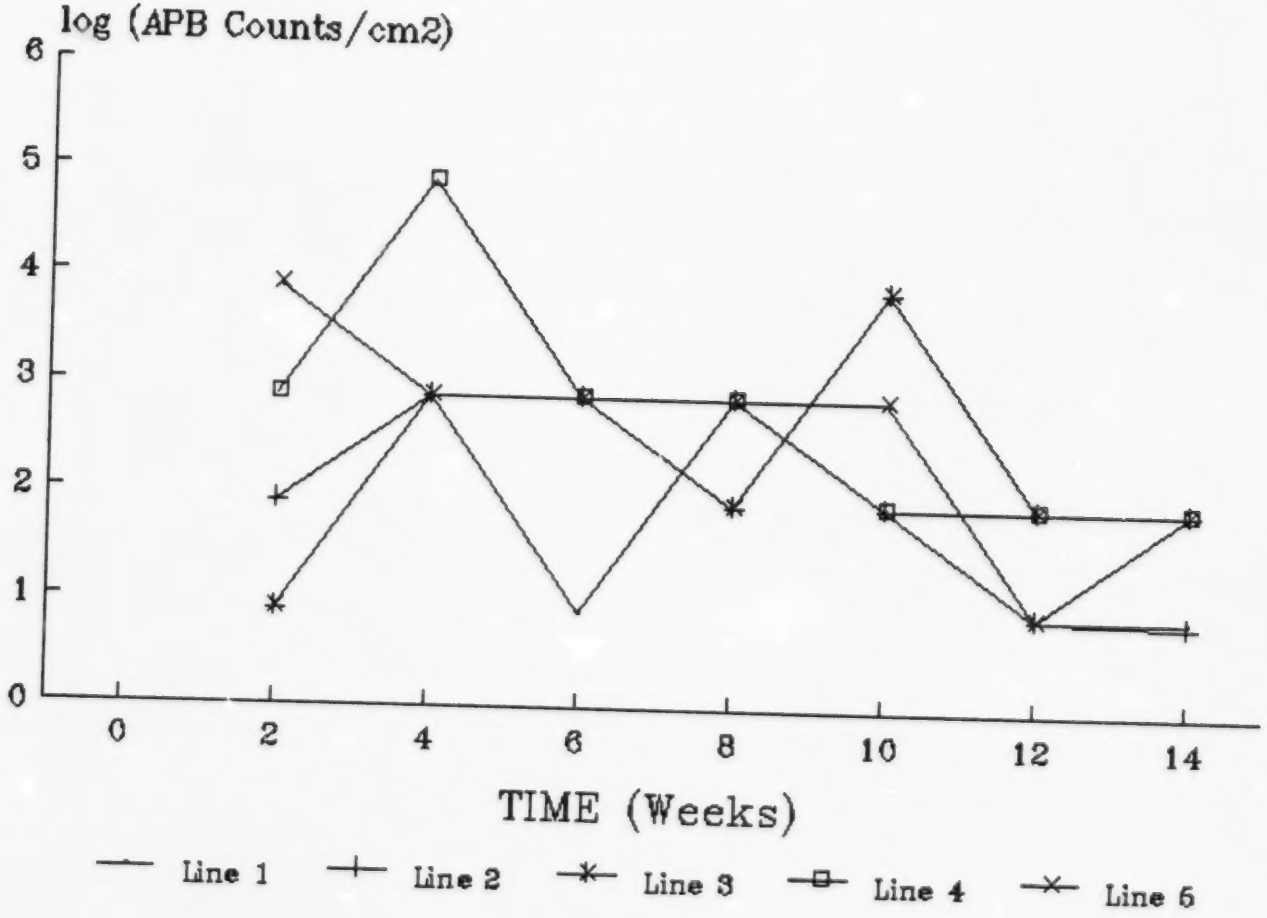

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FIGURE 6. Turnover rates of ^{35}S-labelled sulfate to sulfide before and after biocide treatment during the second experimental run. Biocide treatment started at week 4.

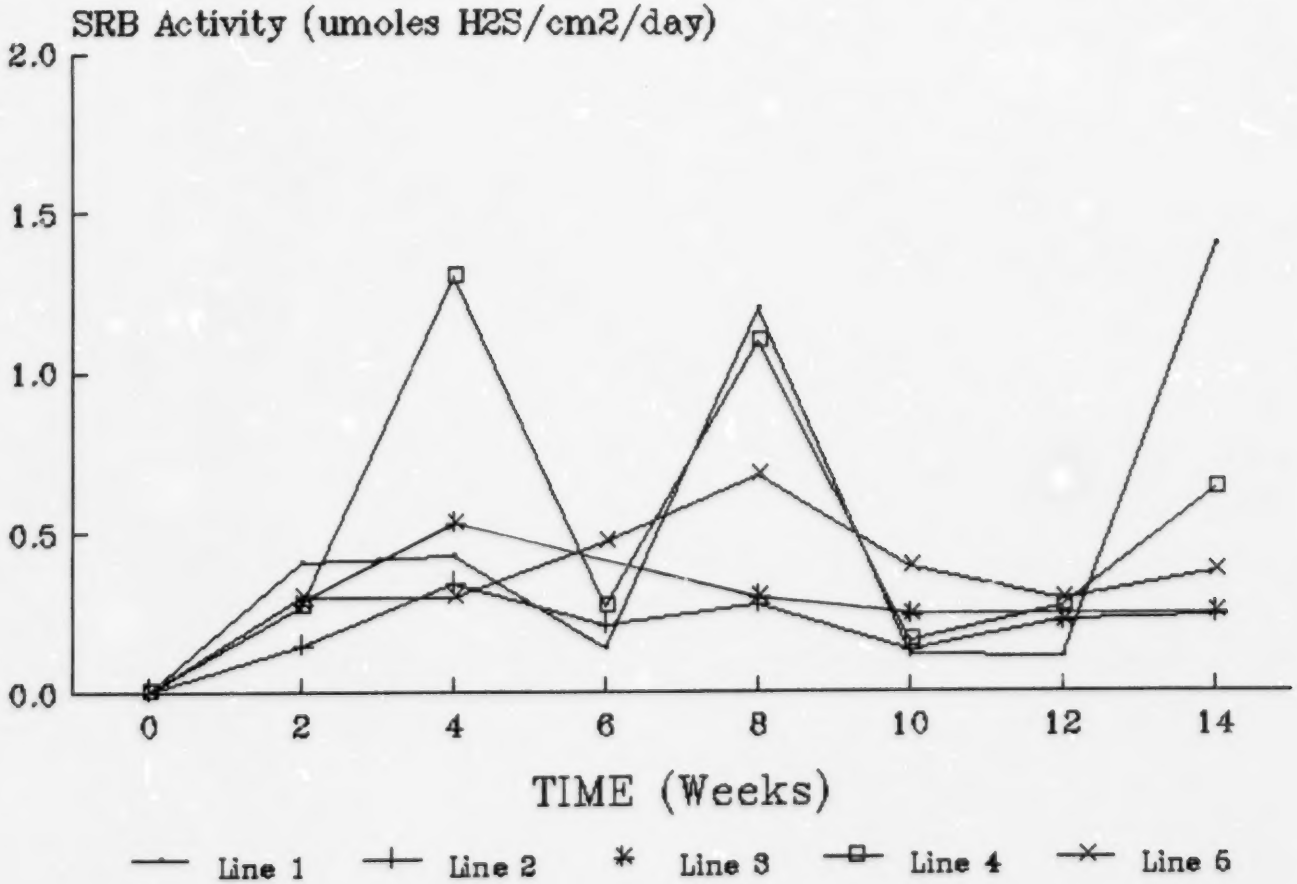

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FIGURE 7. Enhancement of general corrosion rates after biocide treatment in the first experimental run

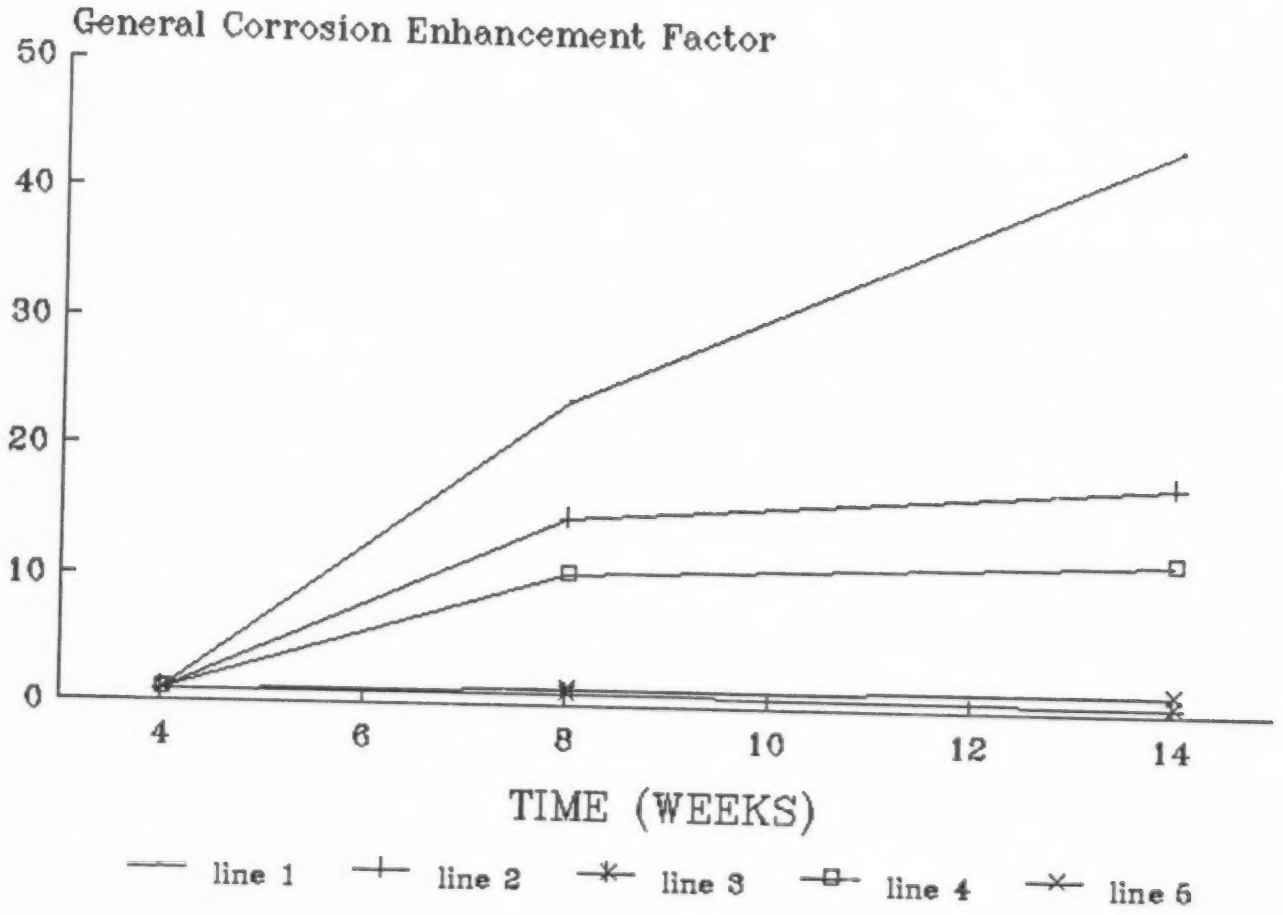

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FIGURE 8. Enhancement of pitting tendency rates after biocide treatment in the first experimental run

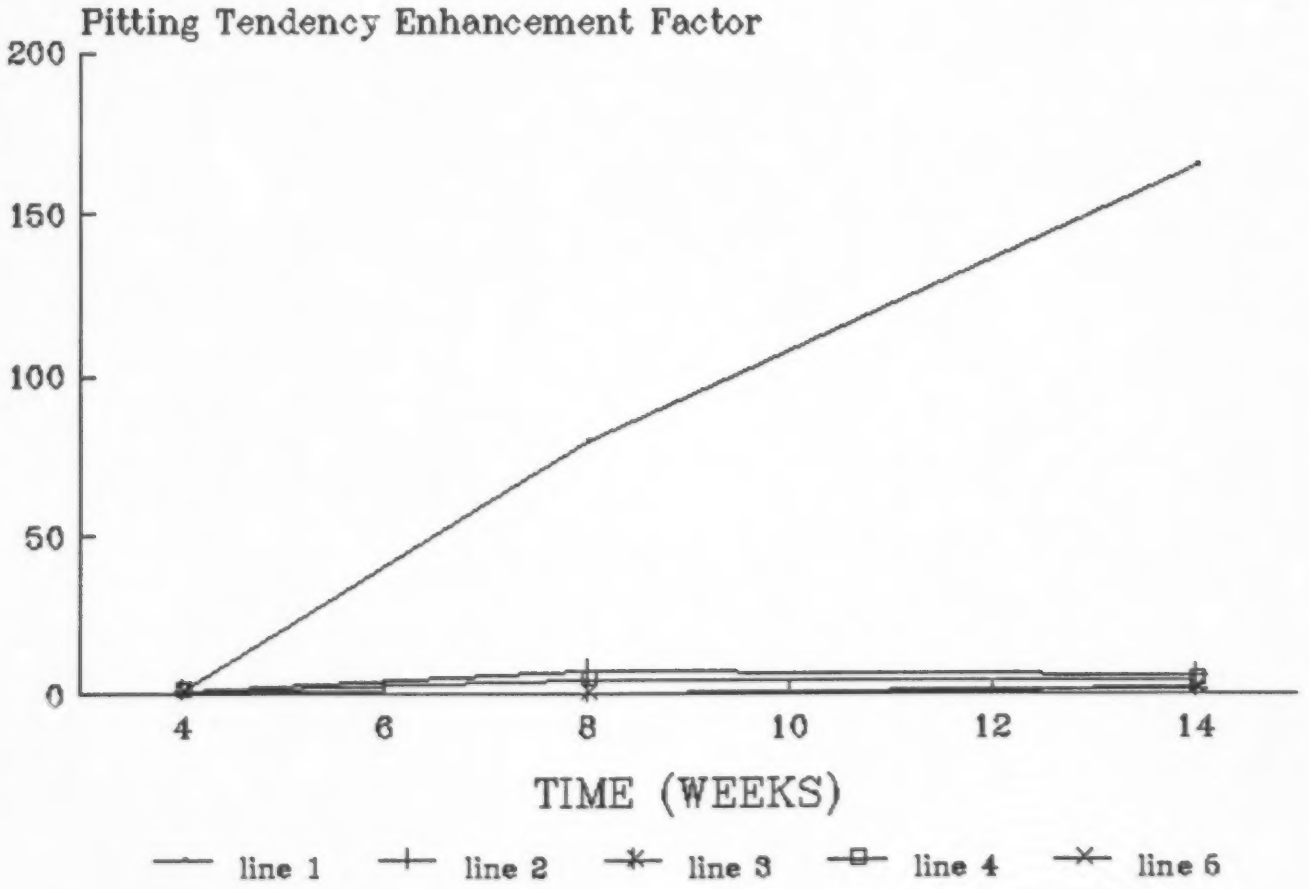

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FIGURE 9. Enhancement of general corrosion rates after biocide treatment in the second experimental run

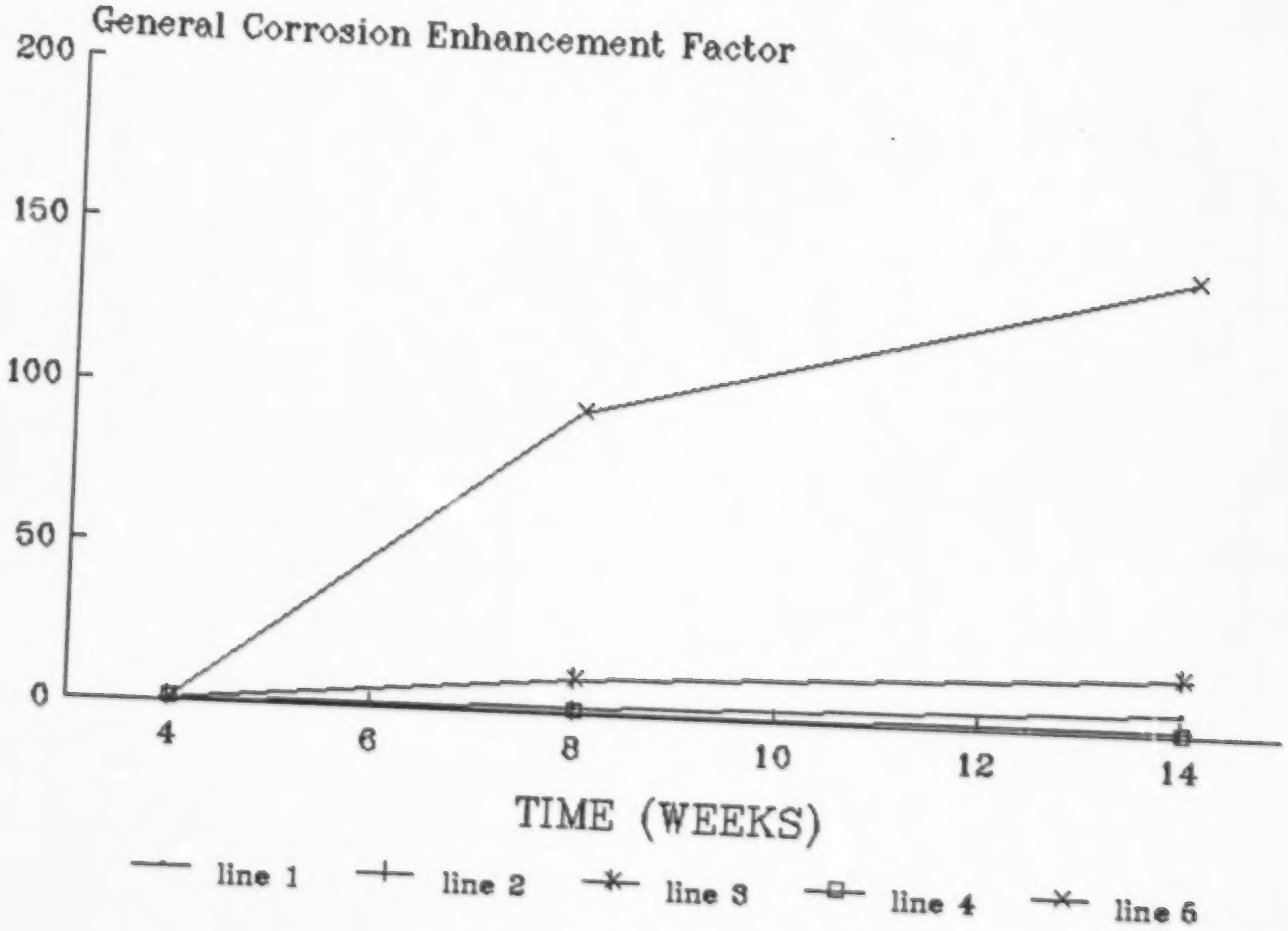

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FIGURE 10. Enhancement of pitting tendency rates after biocide treatment in the second experimental run

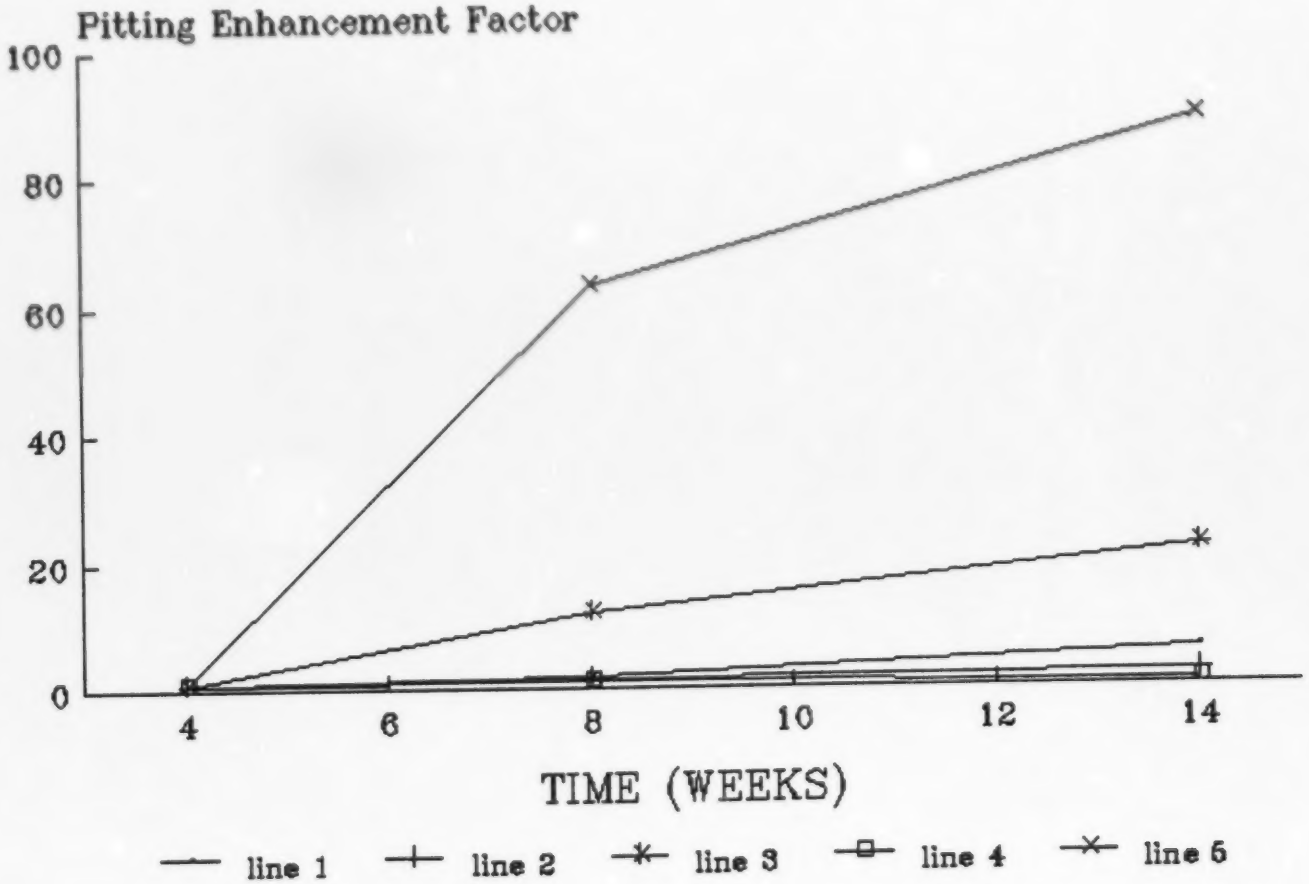

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FIGURE 11. Scatter plot of general corrosion rates and viable sulfate-reducing bacteria counts

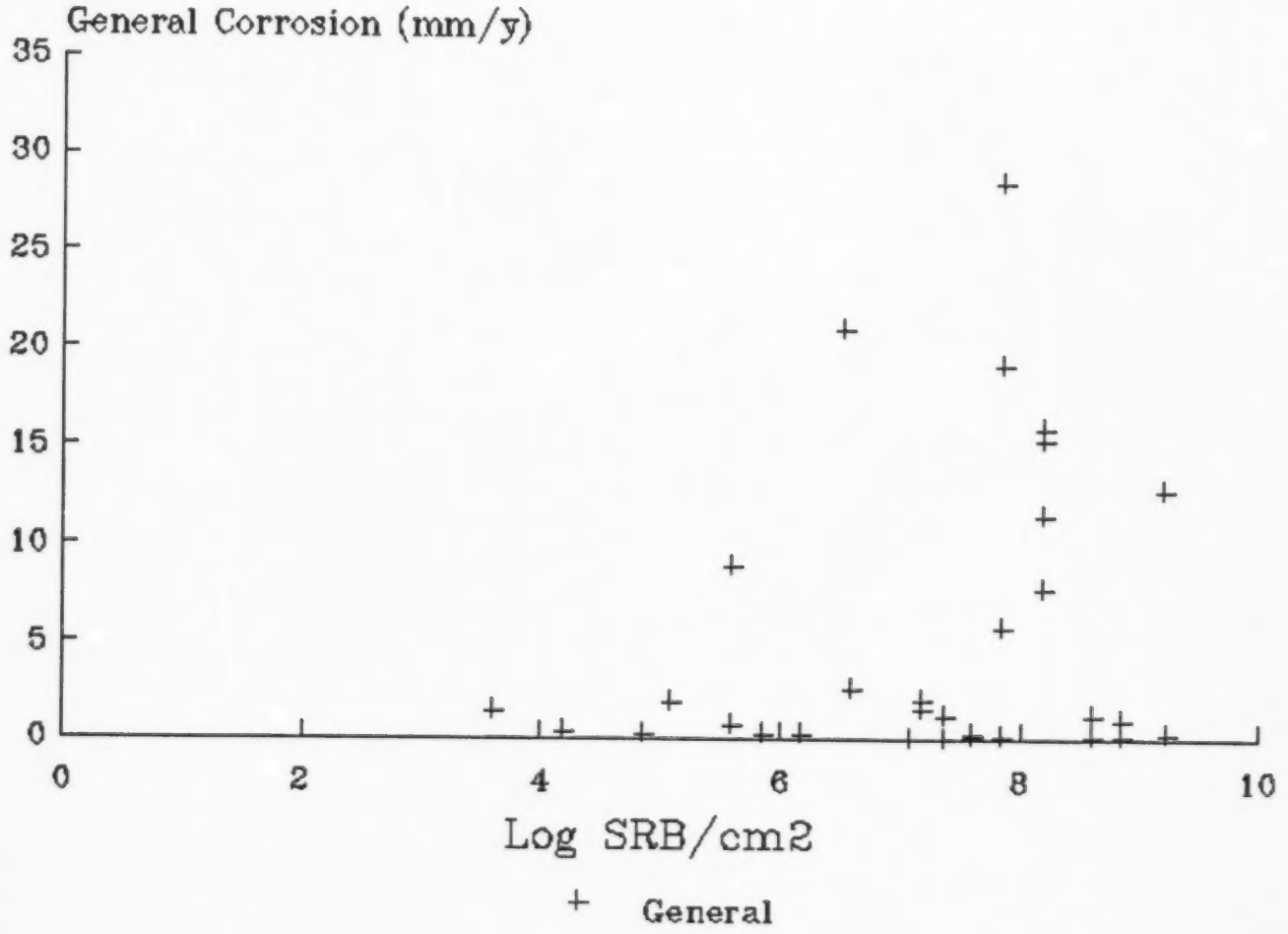

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FIGURE 12. Scatter plot of general corrosion rates and viable acid-producing bacteria counts

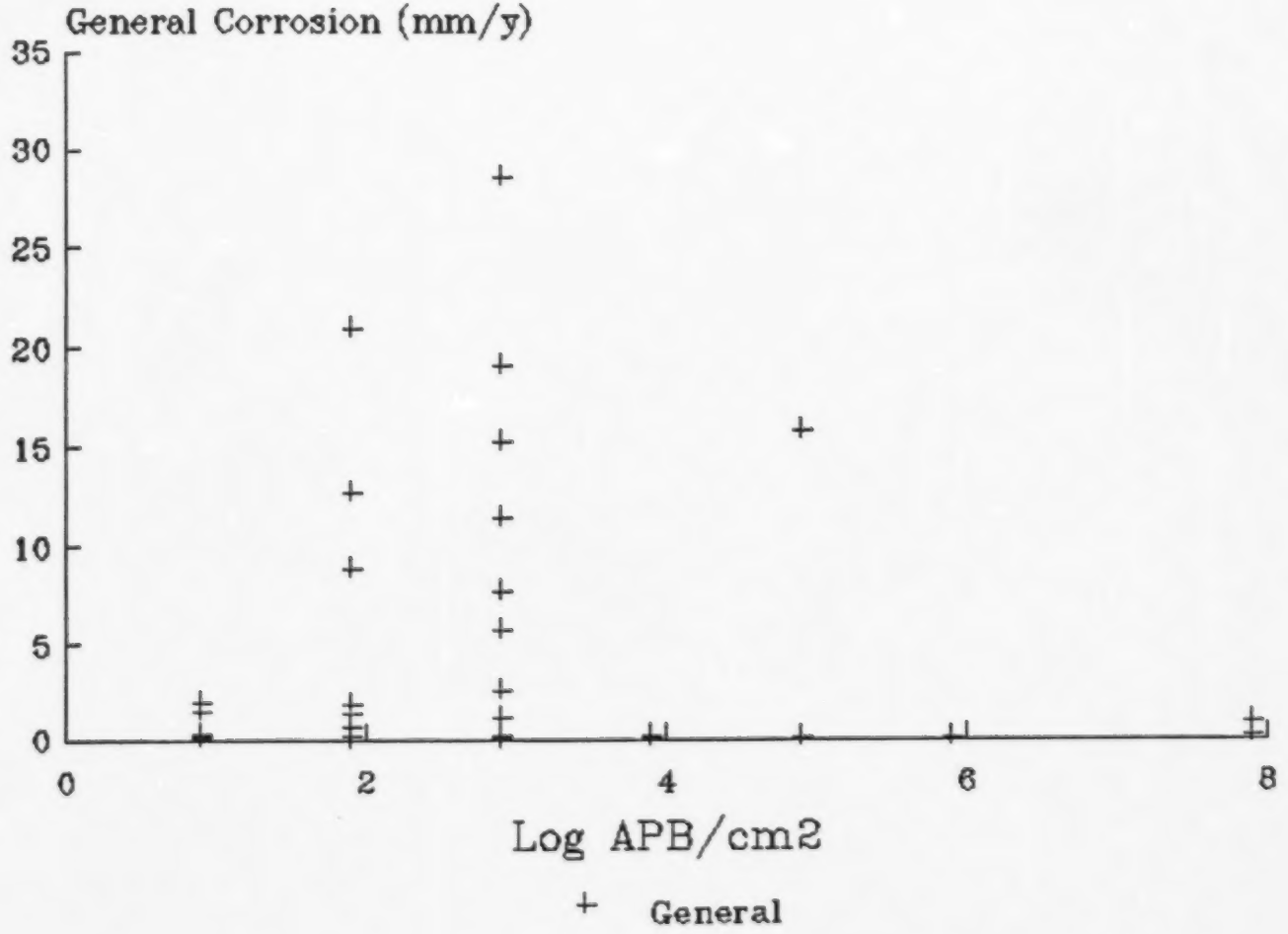

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FIGURE 13. Scatter plot of pitting tendency rates and viable sulfate-reducing bacteria counts

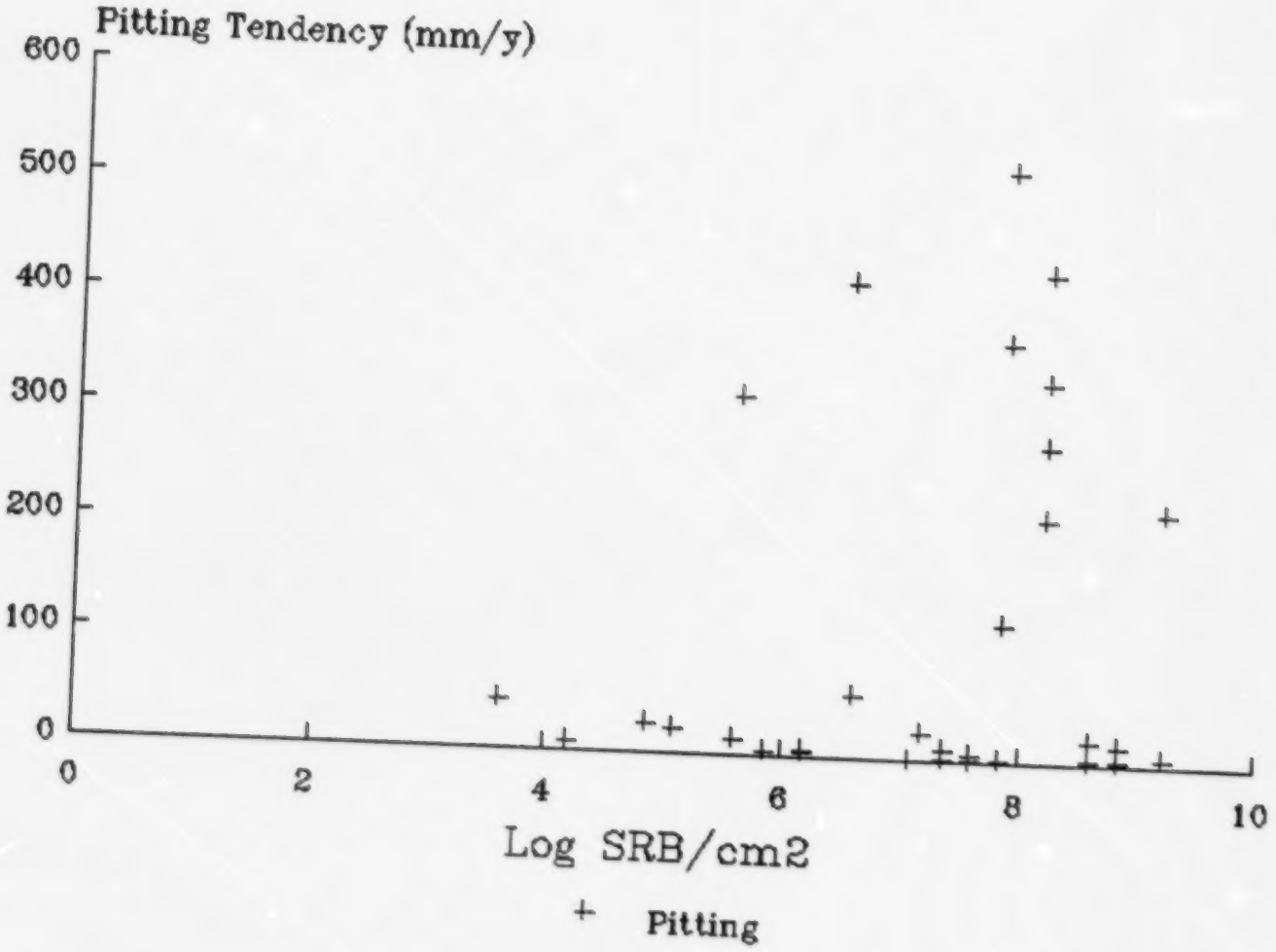

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FIGURE 14. Scatter plot of pitting tendency rates and viable acid-producing bacteria counts

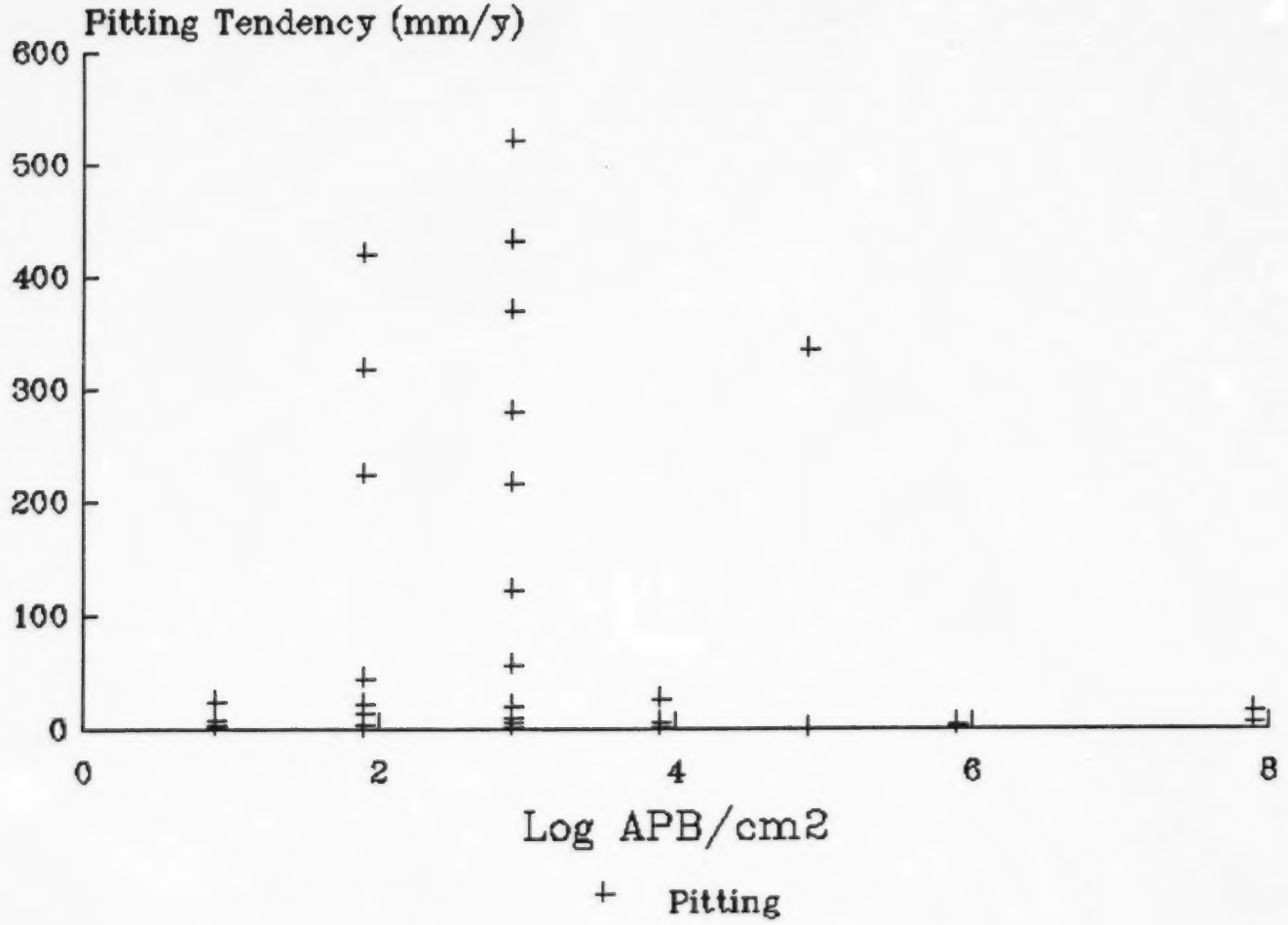

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FIGURE 15. Scatter plot of general corrosion rates and turnover rates of ^{35}S-labelled sulfate to sulfide

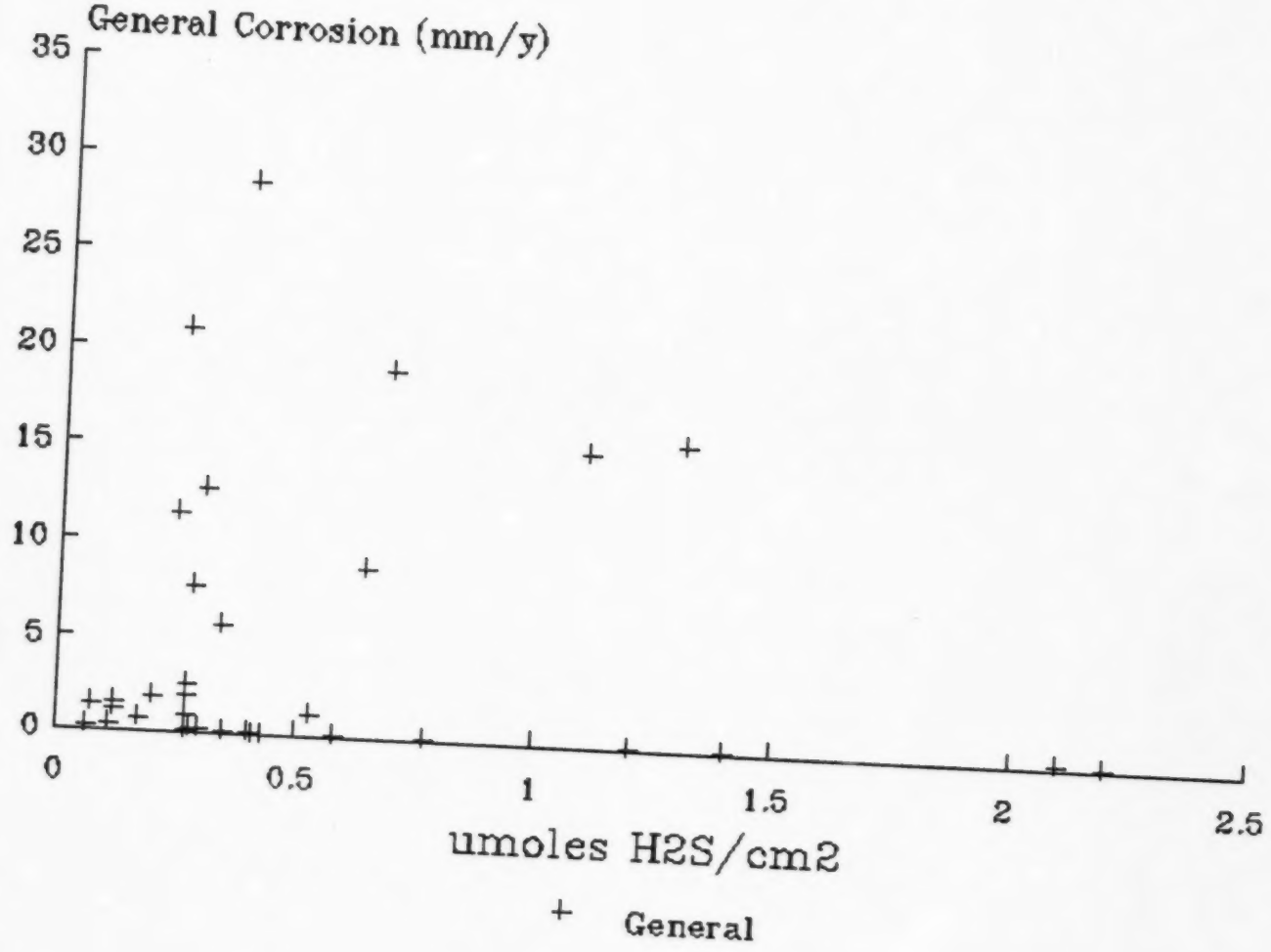

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FIGURE 16. Scatter plot of pitting tendency rates and turnover rates of ^{35}S-labelled sulfate to sulfide

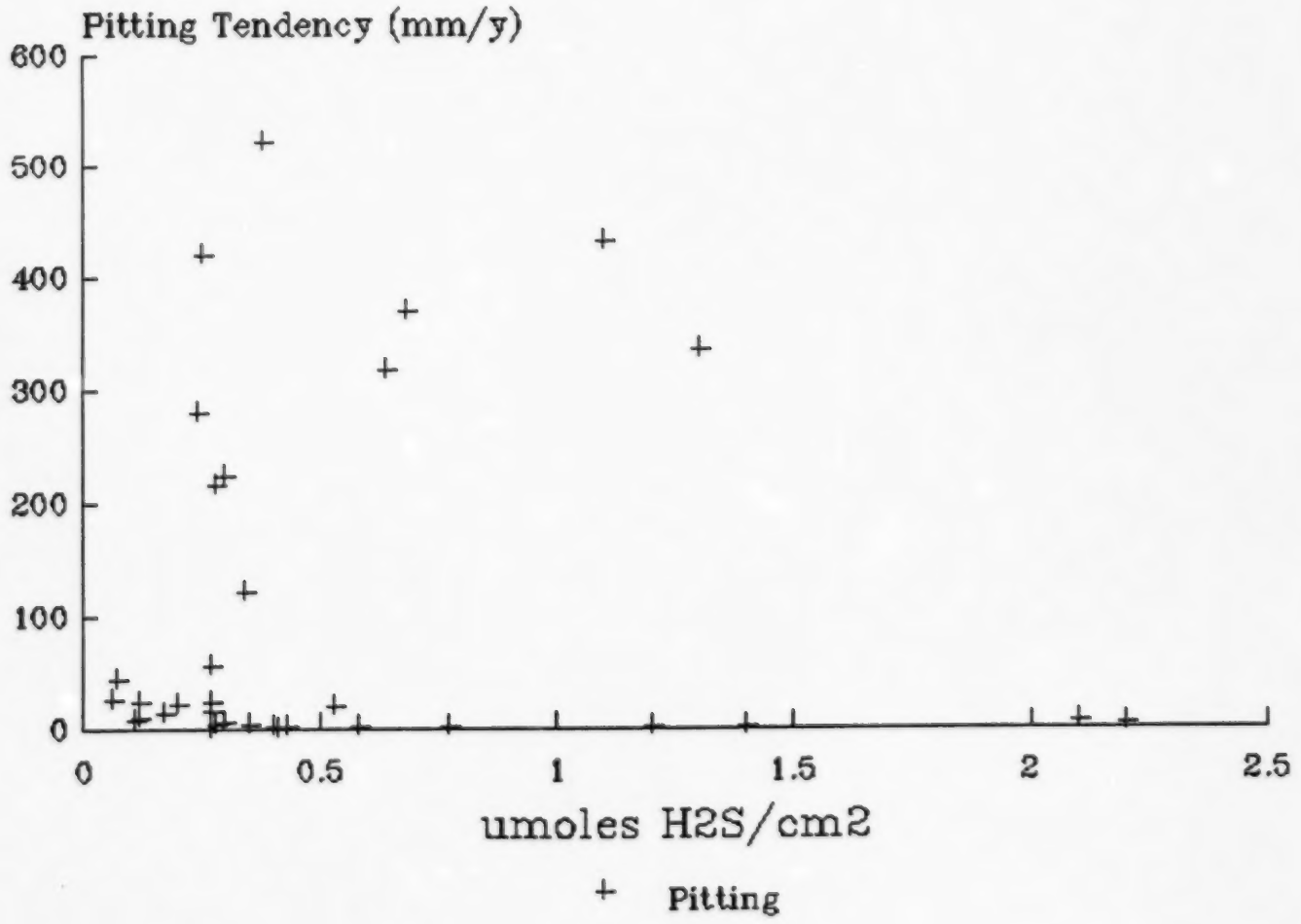

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FIGURE 17. Scanning electron micrograph of surface on an uncoated sample coupon from the field test facility

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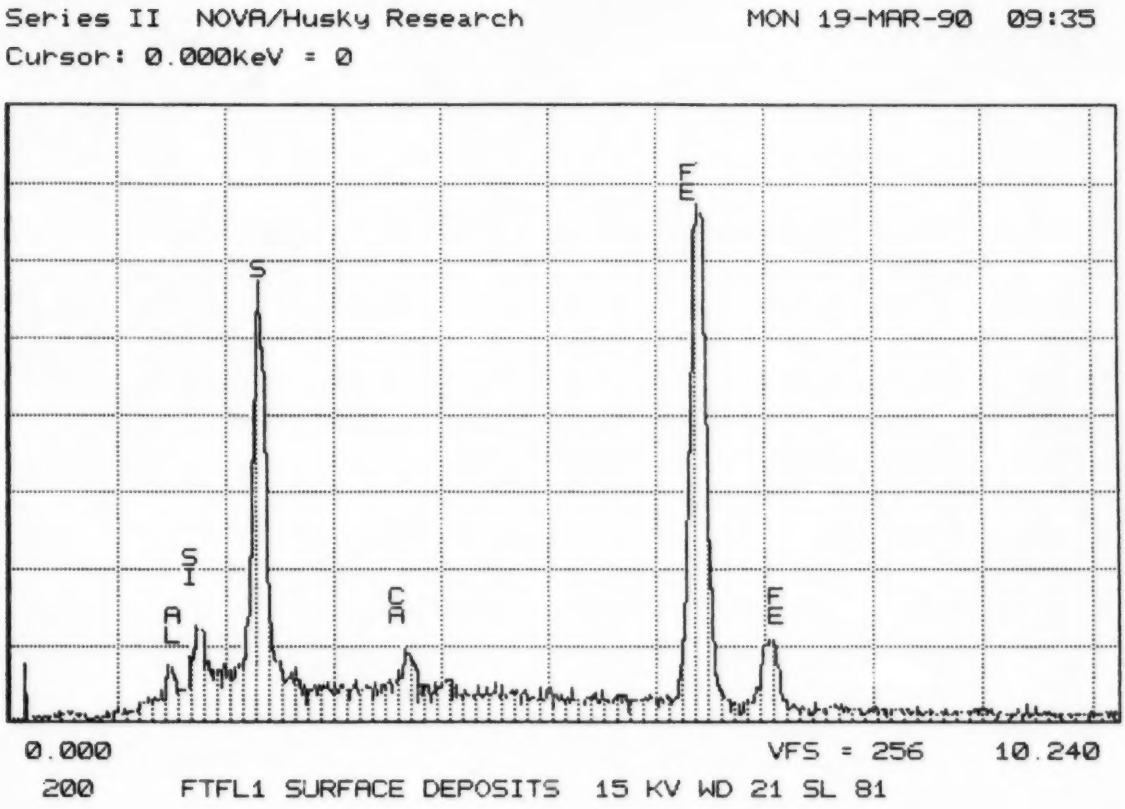

FIGURE 18. Energy dispersive X-ray spectrum from a thin corrosion deposit on the surface of a sample coupon from the field test facility

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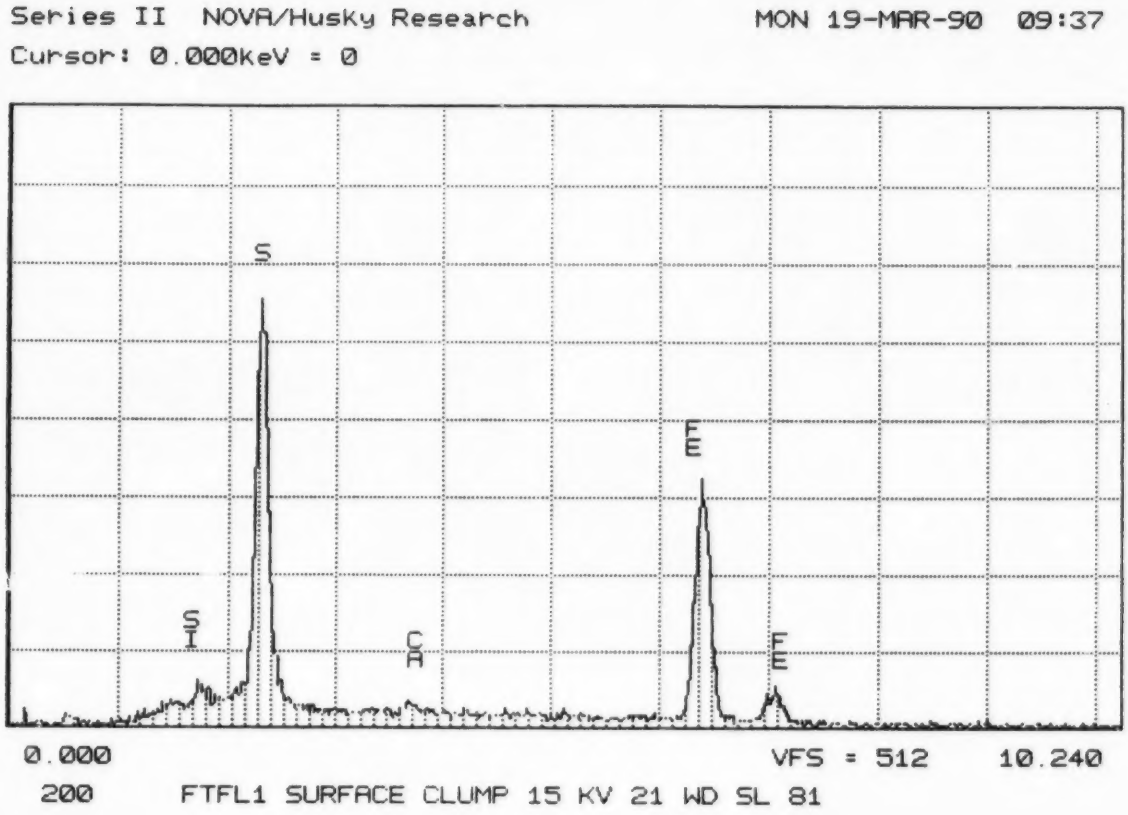

FIGURE 19. Energy dispersive X-ray spectrum from a thick corrosion deposit on the surface of a sample coupon from the field test facility

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FIGURE 20. Scanning electron micrograph of surface on a coated sample coupon from the field test facility showing two distinct textural regions on thick corrosion deposits

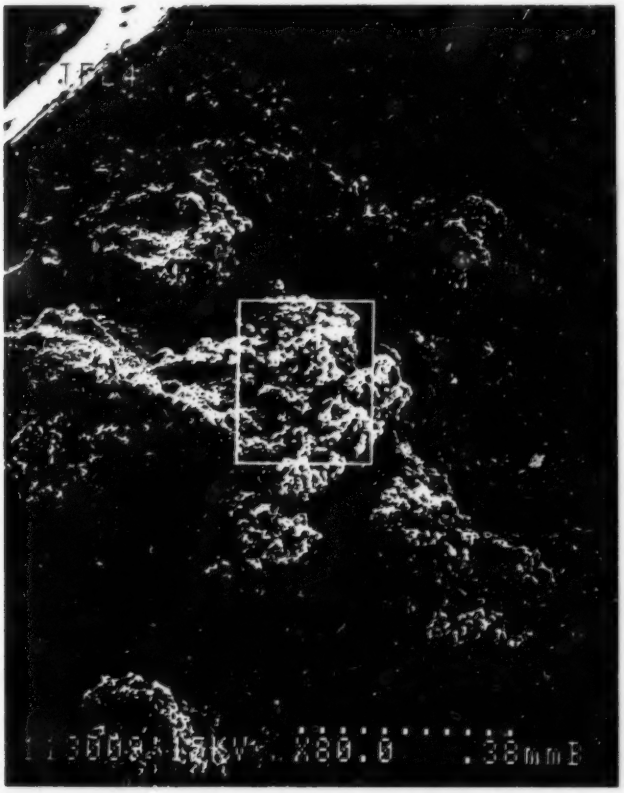

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FIGURE 21. Scanning electron micrograph of bacteria associated with porous regions on large corrosion deposits, (A) vibroid bacteria and (B) filamentous bacteria

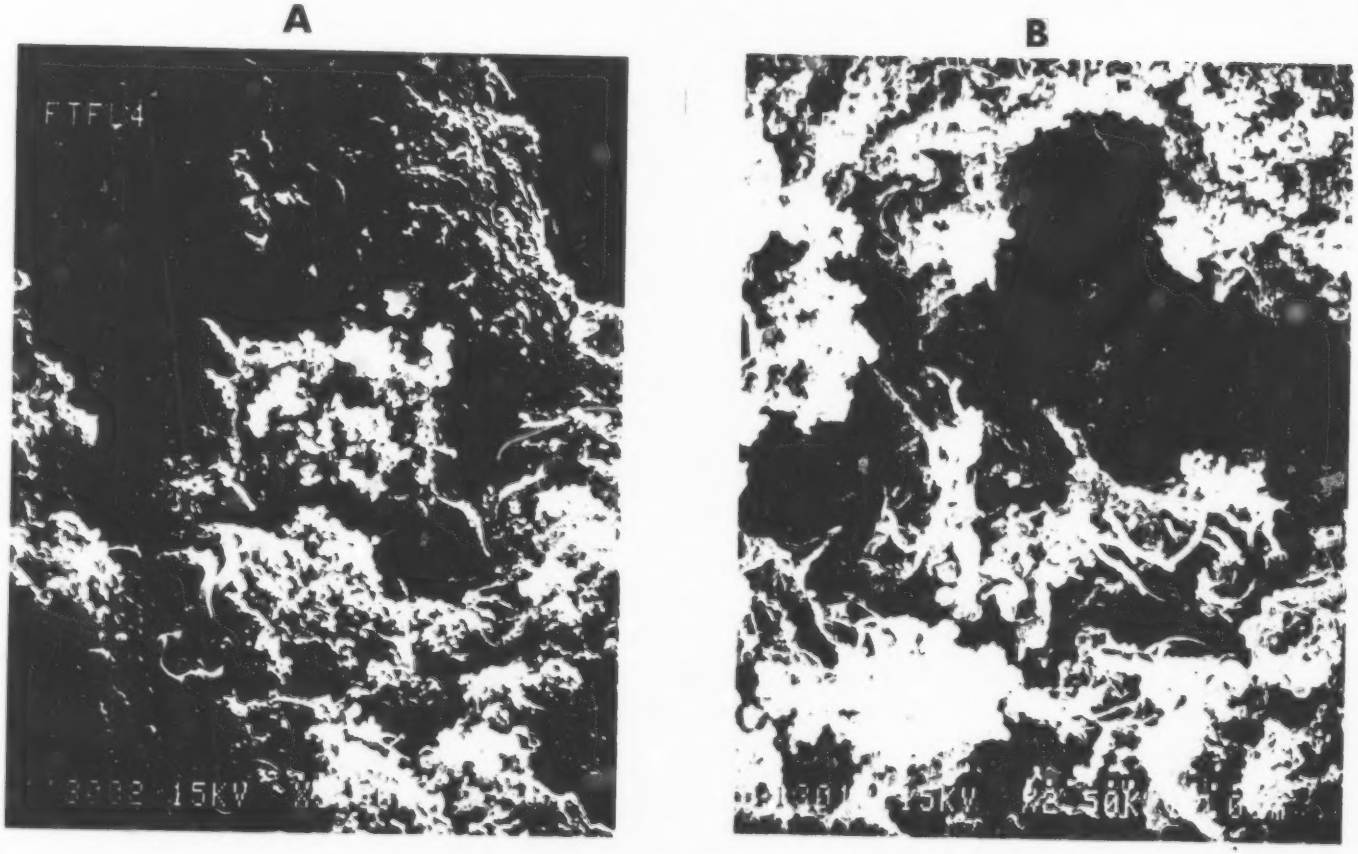

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FIGURE 22. Scanning electron micrograph of bacterial cells in successive stages of mineralization by iron sulfides, (A) cell surface deposits and (B) burial of bacterial cells (arrows).

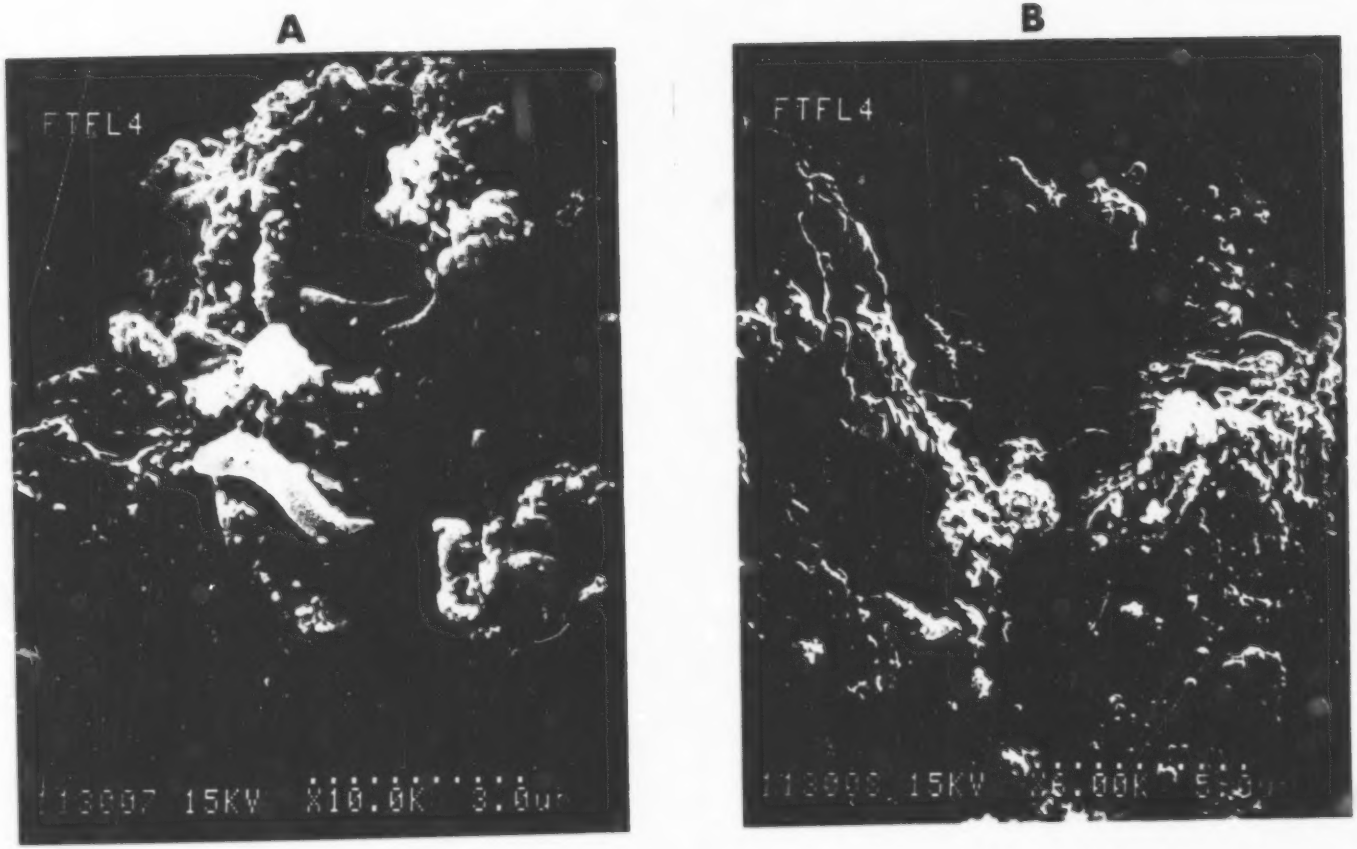

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FIGURE 23. Scanning electron micrograph showing incipient formation of small holes or tunnels (arrows) oriented in the direction of rolling on the surface of a sample coupon from the field test facility, (A) uncleaned surface, (B) cleaned surface and (C) nascent tunnels

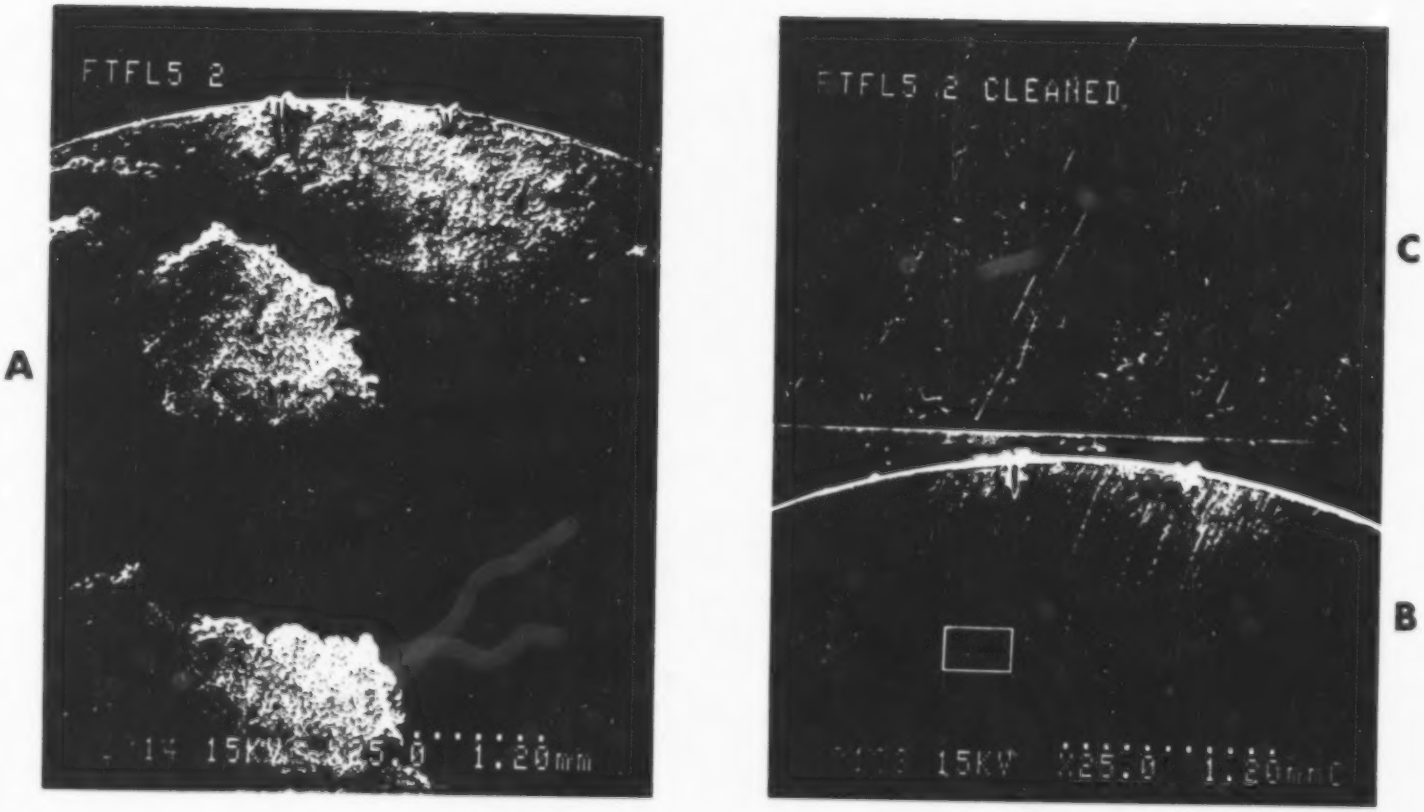

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FIGURE 24. Scanning electron micrograph showing incipient formation of small holes or tunnels (arrows) oriented in the direction of rolling on the surface of a sample coupon from the field test facility, (A) uncleaned surface, (B) cleaned surface and (C) nascent tunnels

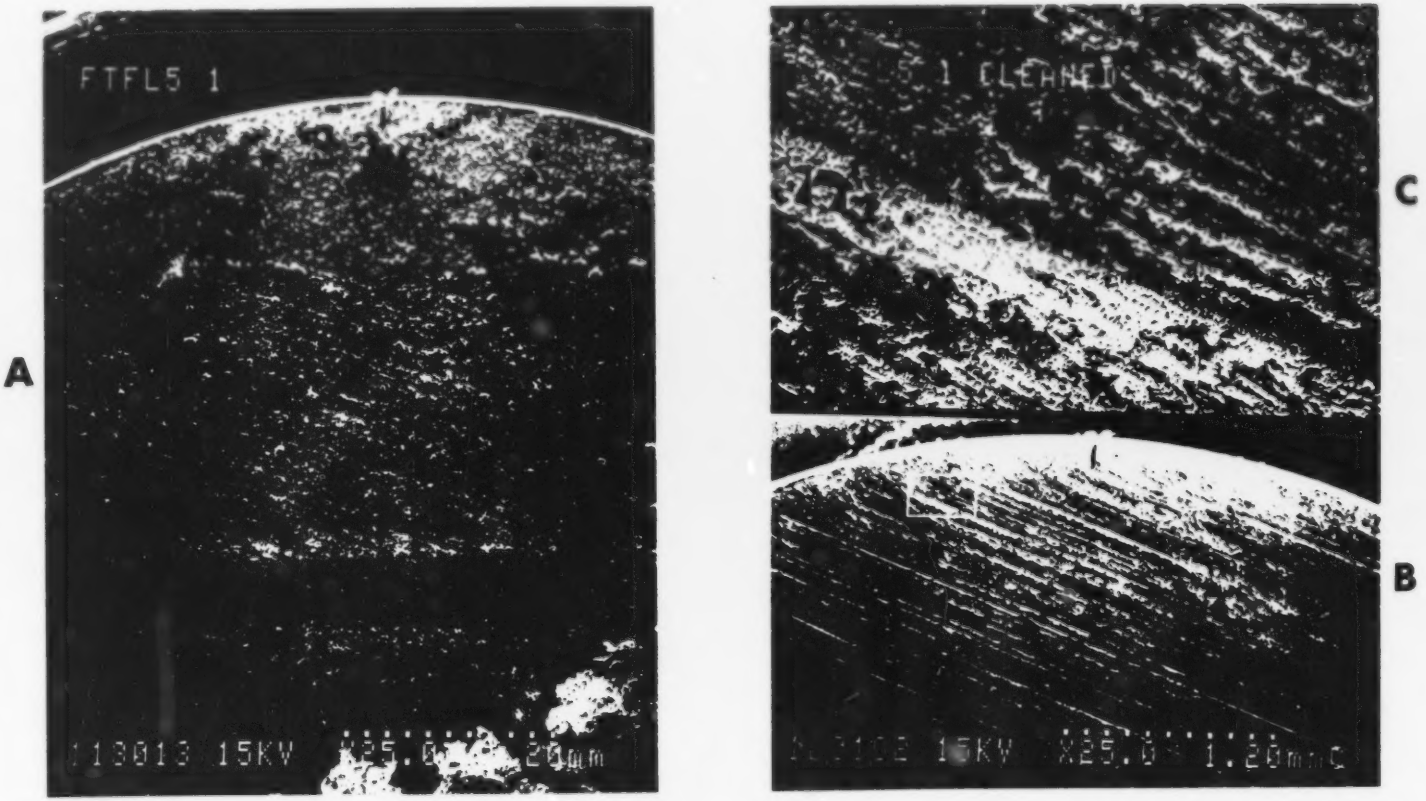

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FIGURE 25. Scanning electron micrograph showing area on the surface of a sample coupon devoid of corrosion deposit, (A) before cleaning, (B) after cleaning and (C) at a higher magnification after cleaning

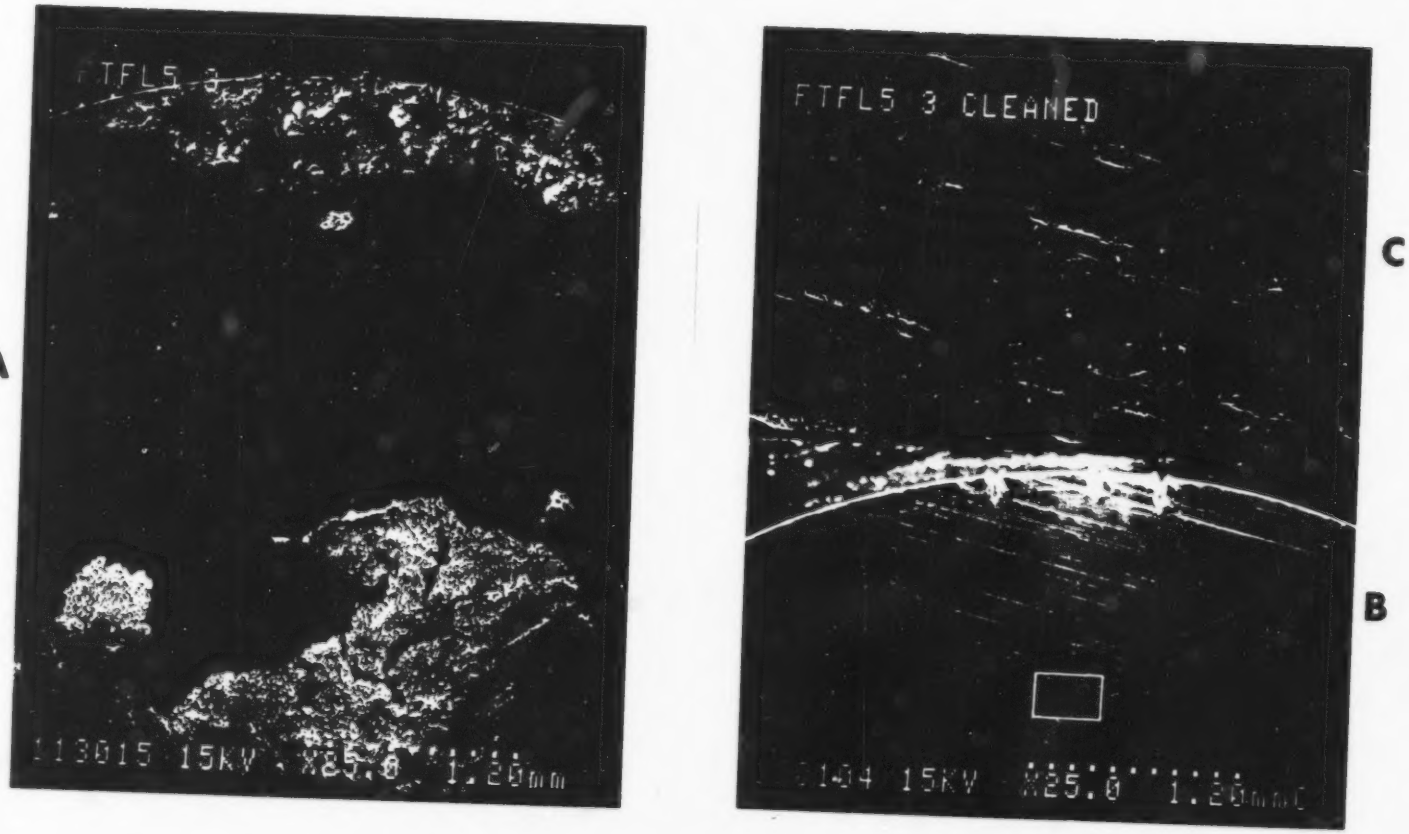

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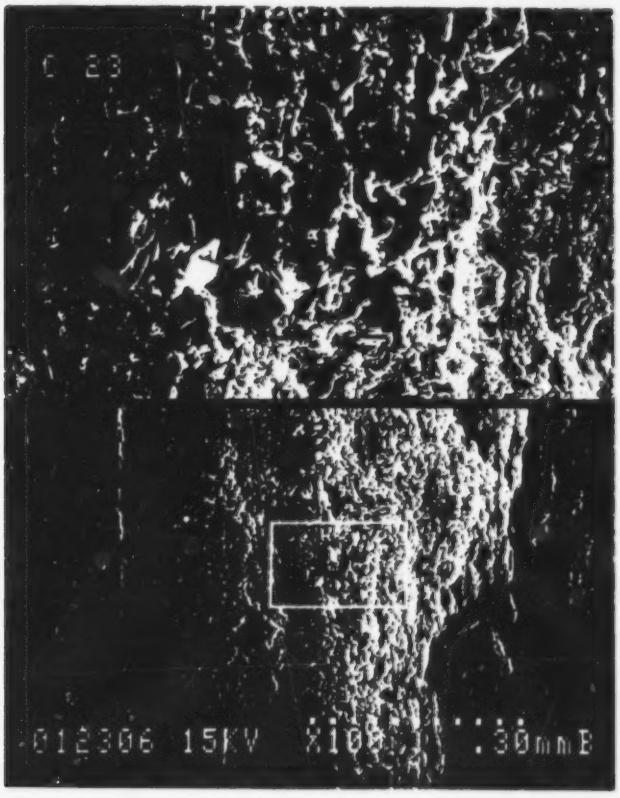
FIGURE 26. Scanning electron micrograph showing tunneling corrosion on a sample coupon previously exposed to a pure culture of *Desulfovibrio vulgaris* NCIB 8303, (A) at low magnification and (B) at a higher magnification



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